LABORATORY STUDIES ON THE SEROLOGICAL PHENOMENA IN SYPHILIS.

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by

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INTRODUCTION.

SECTION A.

I N T R O D U C T I O N.

The problems of immunity to the various infective diseases have proved a fruitful field of scientific study which has yielded results both of fundamental biological importance and also of far reaching practi-:cal application. The recognition of the part played by constituents of the blood serum in protecting the host from invading micro-parasites was one of the early results of immunological study, and the mechanism of the antagonistic effects of serum towards patho-:genic organisms still presents unsolved problems of great physiological and pathological interest. Thus, the study of such serum functions, i.e., of serology, has been ancillary to immunological research in its widest aspects and the demonstration of serological reactions by in vitro tests has exerted a great in-:fluence on the progress of bacteriological and immuno-:logical science. Such studies elicited the fact that the serum of a person or animal possessing an ac-:quired immunity to a particular organism might exert a specific lytic action on this organism both in vivo and in vitro, and the exact analysis of this phenome-:non of bacteriolysis resulted from the elucidation by Bordet in 1898 of the analogous process of serum haemo-:lysis, i.e., the lysis of the red blood corpuscles of a particular species towards whose red cells another animal/

animal had been immunized in a manner analogous to artificial antibacterial immunization. Bordet showed that serum haemolysis is due to two serum principles - a specific antibody or new "substance" generated by immunization, and a normal principle, complement or alexin. The analysis of bacteriolysis revealed an analogous mechanism. Thus, in the process of immunization against a particular organism, a new principle makes its appearance in the serum which, along with the normal serum complement, may exert a specific bactericidal effect. In vitro study of this process has shown that the immune body combines firmly with the homologous bacteria (i.e., the antigen) and that the antigen plus the combined antibody is then highly susceptible to the cytolytic action of the complement, the complement combining with the complex (antigen plus antibody).

Muir (1909) has suggested that the immune body acts by bringing more complement into intimate relationship with the antigen than would otherwise occur, complement having by itself only a relatively weak combining affinity for bacteria. An antibacterial serum may, however, fail to exhibit any bacteriolytic effect but nevertheless contain specific immune substances which unite with the antigen and, as in the former case, lead to the union of a large amount of serum complement. Even apart from antibactericidal immunity, any antigenic substance along with its specific/

specific antibody may bind ("fix, "absorb" or "deviate") complement while the antigen and antibody separately fail to produce any such effect. Such fixation of complement has thus come to be utilised as an in vitro phenomenon indicative of the interaction between an antigen and its specific antibody, fixation being de-:monstrated by the absence of haemolysis on the addit-:ion of the red cells of some appropriate species sensitized with the homologous "haemolytic" antibody.

The high degree of specificity of antibodies for homologous antigen is one of the most striking of biological phenomena. Thus, the species of origin of an exceedingly minute quantity of serum can be identified correctly by means of the complement-fix-:ation antigen-antibody reaction. This specificity has enabled antigen-antibody reactions to be extensive-:ly applied in diagnosis and in the identification of bacteria and has been the basic principle underlying such application of immunology in practical medicine.

The evolution of the complement-fixation reaction as a diagnostic test

Bordet and Gengou (1901) in their original work on the complement-fixation reaction found that while a suspension of Bacillus typhosus exerted only a slight absorptive action on serum complement, if the organisms were first "sensitized" with the homologous immune serum, the amount of complement deviated or fixed was considerable. As their method of demons-:trating/

demonstrating the reaction of complement-fixation has been the prototype of all subsequent application of the phenomenon, some brief reference may be made here to the system they adopted.

The antigens used were bacterial in nature, viz:- Bacillus typhosus, Bacillus pestis and Bacillus proteus and specific antisera were obtained in the usual way. The antigens were used in the form of suspensions in saline of twenty-four hour cultures; the antiserum was heated for half an hour to annul its complement (which is labile at this temperature 56°C.). For test purposes the fresh serum of a normal guinea-pig was used as complement. The haemolytic system employed as an indication of the presence of free complement in the test mixtures consisted of the red blood corpuscles of a rabbit (washed free from serum with saline solution) sensitized with the heated serum (56°C.) of a guinea-pig which had been immunized with rabbit's red cells, i.e., rabbit's red corpuscles plus a specific "haemolytic" antibody.

The technique was as follows:- To 0·4 c.c. of the bacterial emulsion was added 1·2 c.c of inactivated antiserum and 0·2 c.c. of complement. After mixing, the tubes were left at room temperature (20°C.) for several hours. Two drops of the sensitized rabbit's corpuscles were added and it was found that haemolysis did not occur, i.e., the complement had been fixed.

As/

As a control test normal serum was used with the re-:sult that haemolysis occurred in a few minutes, show-:ing that complement had remained "free". The follow-:ing table illustrated the original experiments:-

Tube	Bacterial Antigen.	Antibacterial serum heated 56°C. for half-an-hour.	Guinea-pig's comple-:ment.	Rabbit's red cells plus haemolytic sera (56°C.)	Results.
1	0·4 c.c.	1·2 c.c.	0·2c.c.	2 drops sensi-tized rabbit's blood.	No lysis.
2	0·4 c.c.	Normal serum 1·2 c.c.	0·2c.c.	2 drops sensi-tized rabbit's blood.	Complete lysis.
3	. . . .	Antibacterial serum 1·2c.c.	0·2c.c.	2 drops sensi-tized rabbit's blood.	Complete lysis.
4	. . . .	Normal serum 1·2 c.c.	0·2c.c.	2 drops sensi-tized rabbit's blood.	Complete lysis.
5	0·4 c.c.	Antibacterial serum 1·2c.c.	. . . .	2 drops sensi-tized rabbit's blood.	No lysis.
6	0·4 c.c.	Normal serum 1·2 c.c.	. . . .	2 drops sensi-tized rabbit's blood.	No lysis.

Gengou (1902) next showed that proteins in solut-:ion, e.g., serum and milk, could stimulate production of complement-fixing antibodies, in addition to pre-:cipitins. Moreschi (1905) showed that when an anti-:serum and its homologous protein were mixed precipi-:tation occurred and the complement disappeared, be-:lieving that it was carried down mechanically in the precipitate./

precipitate. This explanation appeared to throw new light on the Bordet-Gengou phenomena and Gay (1905) confirmed much of Moreschi's work. Neisser and Sachs (1905) improved on the existing methods and were able to devise a process by which small traces of protein could be differentiated by means of the complement-fixation test though the precipitation test failed in this respect. Widal and Lesourd (1901) found that in enteric fever the Bordet and Gengou reaction with Bacillus typhosus and the patient's serum was positive at an earlier date than the corresponding agglutination reaction. Since these observations were made soon after Bordet and Gengou's original work, they probably represent the first direct and practical application of the complement-fixation test in diagnosis.

The original Wassermann reaction

It had been shown that if bacteria were macerated in water or saline solution specific antigens could be extracted, e.g., if the spleen from a case of enteric fever is extracted with normal saline solution, an antigen is obtained which, in conjunction with an anti-typhoid serum, produces fixation of complement. Wassermann, Neisser and Bruck in 1906 first applied the Bordet-Gengou phenomena to the diagnosis of syphil-:is. They immunized monkeys with aqueous extracts of human chancre, condylomata and syphilitic placenta; and/

and the sera of these animals when mixed in vitro with the extracts were found to give the complement-fixation reaction. The early work showed that the reaction possessed a high degree of specificity as the immune monkey serum did not react with extracts of normal organs or normal monkey serum failed to show any effect with extracts of syphilitic organs. For further applications of the reaction in diagnosis these workers prepared as antigen an aqueous extract from syphilitic foetal liver which contains numerous spirochaetes. They tested this antigen in the complement-fixation reaction along with the serum of a known syphilitic person and guinea-pig's complement, and after these reagents had been allowed to interact, a complement-free haemolytic system was added (sheep's red cells plus a homologous haemolytic serum). As they anticipated, haemolysis did not occur, indicating the fixation of the complement. Detre (1906) obtained similar results, though working independently, which he published 14 days later. Marie and Levaditi (1907), Weil and Braun (1907) and others, however, found that the same deflection or fixation of complement took place when extracts of normal liver and extracts of certain tumours, etc. were used instead of syphilitic liver extracts. In turn, Wassermann, Porges and Meier (1907-1908) later showed that the active substances in the extract of liver were soluble in alcohol. These substances proved to be lipoid in nature and/

and Wassermann and his co-workers finally accepted the view that the substance responsible for the phenomenon was lecithin. It was shown by Landsteiner, Müller and Pötzl (1907) that an alcoholic extract of normal non-syphilitic organs might likewise furnish an antigen which would give a reaction with syphilitic serum. Browning and Mackenzie (1910) found that the addition of cholesterol to lecithin caused an increase in the amount of complement absorbed in the presence of syph-:ilitic serum but this was practically negligible in the presence of normal serum under the same conditions. Cholesterol similarly increases the reacting power of many alcoholic tissue extracts and cholesterolized heart extracts find most favour with modern serological workers. The biological syphilis reaction seems to depend on the fact that syphilitic serum in the pres-:ence of certain lipoid constituents leads to the ab-:sorption of an amount of complement in excess of the total of the amounts absorbed by the serum and the tissue constituents by themselves.

The Wassermann reaction as at present applied in the Diagnosis of Syphilis.

When the Wassermann reaction was first introduced by Wassermann, Neisser and Bruck (1906) it was consid-:ered to be a fixation of complement by syphilis ambo-:ceptor and extract of spironema pallidum contained in the syphilitic tissues employed for the preparation of antigens,/

antigens, that is to say, a direct application of the Bordet-Gengou phenomenon. When it was found that antigens for this complement-fixation reaction could be prepared from non-syphilitic tissues, the whole nature and mechanism of the reaction in syphilis became sharply differentiated from the other complement-fixation reactions because the reaction could no longer be regarded as biologically specific. Noguchi (1912), employing antigens from cultures of the spironema pallidum, found that, whereas certain long standing or treated cases of syphilis yielded positive results with these antigens, the reactions were uniformly negative when lipoidal extracts were used. Moreover, in primary and secondary syphilis the reactions with spironema antigens were also negative, whereas with lipoidal extracts they were uniformly positive. He concluded that in syphilis there is produced a true antibody that reacts specifically with spironema antigen, in addition to a lipoidotropic "reagin", which reacts with lipoidal extracts, and whereas the latter indicates activity of the infecting agent, the former is an indication of the defensive activity of the infected patient. Craig and Collins (1912) used alcoholic extracts of spironema pallidum, spironema pertenue and spironema microdentium and found positive results in all stages of syphilis. Kolmer, Williams and Laubaugh (1913) used aqueous and alcoholic extracts of spironema cultures/

cultures and confirmed the result of Craig and Nichols, but found that the watery extracts yielded better re-:sults than the alcoholic extracts, both being inferior to those obtained with the alcoholic tissue extracts. The true nature of the Wassermann reaction may not yet have been determined but it is recognised that lipoid-:al extracts, normal and syphilitic sera may individ-:ually fix small amounts of complement while a mixture of an extract and syphilitic serum is capable of fix-:ing large amounts.

Antigen Function

At the present time the alcoholic extract of a normal organ, with the addition of cholesterol, is mostly used as an antigen for the Wassermann reaction. Sachs and Rondoni (1908) have pointed out that the mode of preparation of the emulsion has a marked effect on its antigenic value. They have shown that the antigenic property of lecithin, with sodium oleate is much greater than that of either of those substances by itself. The essential characteristics of an effic-:ient antigen may be stated to be, (1) very slight haemolytic action, (2) a uniform and almost negligible anticomplementary effect by itself, (3) the power of causing the combination of a large amount of comple-:ment in the presence of syphilitic serum, (4) the power of absorbing no more than the sum of the amounts absorbed/

absorbed by serum and by extract alone in the case of a normal serum and (5) the power of being a delicate reagent for the detection of certain substances induced in a patient's serum as a result of syphilitic infect-:ion. Kyes and Sachs (1903) have pointed out that the haemolytic action of lecithin is diminished by the addition of cholesterol. They found that an emulsion of lecithin-cholesterol by itself scarcely inhibits complement to any greater degree than does the lecith-:in alone. Indeed, the increase of inhibitory effect in complement due to the cholesterol is almost negig-:ible. Iscovesco (1908) and Pighini (1909) attribute to cholesterol by itself a powerful anticomplementary action, but cholesterol does not increase the inhibit-:ory action of lecithin on complement. In the Wasser-:mann reaction the effect of the addition of cholest-:erol to the lecithin is most striking in its effect on the amount of complement absorbed in the presence of syphilitic serum.

Serum Function

It is well known that fresh guinea-pig's serum causes lysis of ox blood corpuscles, an action which is due to small amounts of natural immune body and comple-:ment. It has been shown that immune body at 0°C. becomes anchored by the blood corpuscles (at this temperature complement does not combine). If, then, serum treated with blood corpuscles at 0°C. is centri-:fugalized, all the complement is found in the fluid and/

and the immune body is bound to the corpuscles. The sera of normal persons which have not been heated may give a positive reaction. Heating diminishes the reacting power of a syphilitic serum, and it should not be kept in contact with the clot, otherwise it has greater deviating power -- becomes anticomplementary. An unheated serum, in the presence of antigen is anti-:complementary; this is removed by heating. Bacter-:ial contamination causes a serum to become anticomple-:mentary. Sera which have been heated at 57°C. for an hour do not acquire an anticomplementary action. It is well recognised that when a patient is undergoing antisyphilitic treatment the serum may give a negative reaction. Craig and Nichols (1912) have shown that during an alcoholic bout the serum may be negative. It may give negative results in anaesthesia and in fever. Serum should be clear and free from corpuscles and it should be heated for half an hour at 55°C. be-:fore the test, though unheated serum may increase the sensitiveness. Serum is heated to inactivate the natural complement, to remove thermolabile anticomple-:mentary substances and to remove the possibility of proteotropic non-specific reactions. A control with-:out antigen is included in the test to discover any inhibitory action of the serum itself upon the comple-:ment. Post-mortem serum is anticomplementary. When serum and antigen are mixed before the complement is added, the sensitiveness is increased. Gilmour (1924)/

(1924) has studied the optimum amount of serum for the Wassermann reaction and arrived at the following con-:clusions:-

(1) If the amount of serum is increased, it is necessary to increase the amount of complement to pro-:duce lysis.

(2) Large amounts of normal serum do not give a positive reaction, that is to say, the amount of complement which is inhibited by the serum along with the antigen is not more than the sum of the amounts inhibited by the serum and antigen separately.

(3) In the case of syphilitic sera there is an optimum amount; thus, with 0·05 c.c. of serum the amount of complement fixed along with the antigen is greater than when 0·01 c.c. of serum is used, but when 0·5 c.c. of serum is used, the increased inhibition due to the serum by itself tends to obscure the sign-:ificance of the result. If a serum is tested which is rich in natural sheep immune body and an alcoholic extract of guinea-pig's heart, such a serum will pro-:duce complement-fixation irrespective of its being derived from a case of syphilis (Taniguchi, 1920). The optimum amount of serum to be used in a test de-:pends on the particular syphilitic antigen which is used.

METHODS

Many different systems have been advocated for carrying out the diagnostic test. The following methods have been practically applied by the writer :-

(1) The methods recommended by the Medical Research Council.

(a) Harrison.
(b) Browning.

(2) The method used in the University of Edinburgh, as recommended by Mackie.

(3) The method of Bordet and Ruelens.

(4) The method of Neymann and Gager.

(5) The method of Boas.

For most of the studies recorded in this thesis, the method used in the Bacteriological Department, Edinburgh University has been employed, and has been found, as the results show, a highly sensitive tech-:nique and entirely satisfactory from the diagnostic standpoint. It is not intended to enter here into any detailed comparison of various methods but some comparison of certain different types of antigens has been made.

(1) The cholesterolized sheep's heart extract used in the Bacteriological Department of the University of Edinburgh.

(2) Cholesterolized ox heart extract.

(3) Bordet and Ruelens' antigen.

(4) Neymann and Gager's antigen.

(1)/

(1) Method used in Edinburgh University.

Preparation of Antigen.- The muscle of a sheep's heart, freed from fat and vessels, was put through a mincing machine, finely and thoroughly ground with sand and then extracted with 96 per cent. alcohol (20 grams of heart to 100 c.c. of alcohol); the extract was shaken from time to time and finally filtered. It was saturated with cholesterol, being dissolved with heat at 37°C. and vigorously shaken and allowed to stand for some days before use. Antigen for the test was made up in a 1 : 12 dilution with 0·85 per cent. normal saline by slow admixture so as to form a turbid suspension. Complement was obtained from a guinea-pig, killed the previous night, the serum being allowed to separate from the clot in the ice chest.

Technique.- The M.H.D. of complement for 0·5 c.c of sensitized blood suspension was estimated by a pre-:liminary test and adding 0·04, 0·06, 0·08 and 0·1 c.c. (1 : 10 dilution) of complement to 4 tubes containing 0·5 c.c. of sensitized blood. The patient's serum was inactivated at 55°C. for half an hour. Three cubic centimetres of red blood corpuscles (which had been washed three times with 0·85 per cent. saline solution) were suspended in normal saline solution, made up to 100 c.c. and sensitized with not less than 5 doses of a rabbit v. ox haemolytic immune body; this/

this formed the haemolytic system. Fixed amounts of antigen (0·5 c.c.) and patient's serum (0·05 c.c.) were tested with varying amounts of complement (2, 4, 6 and 10 doses). Positive and negative controls were always included among the sets of sera to be tested. Serum and antigen controls were also included. Read-:ings were made after incubation for half an hour and also generally 12 - 18 hours later.

(2) Antigen of Bordet and Ruelens.

Bordet and Ruelens (1919) mince the heart muscle of a calf, care being taken not to express the juice; 100 grams of this are shaken with 125c.c. of 94 per cent. alcohol, which serves to coagulate the proteins without having any appreciable solvent action on the lipoids. The mixture is left for some days at room temperature and then filtered; the tissue, after draining, is spread out on glass and placed for about a day in the incubator at 37°C. The dry material is mixed with 200 c.c. of acetone and left for about a week at 18-20°C. The mixture is then filtered; the residue is allowed to stand for a day in a little more acetone in order to remove the last traces of soluble substances, and the acetone is again filtered off. The residue is then kept in the incubator for some hours to remove acetone and is then allowed to stand for 8 or 10 days at 20°C. in 200 c.c. of 94 per cent. alcohol; the filtrate from this constitutes the antigen./

antigen.

(3) Antigen of Neymann and Gager.

Neymann and Gager (1917) recommend guinea-pig or ox heart, but in view of the objection raised by Tani-:guchi (1920) the alternative (ox heart) was used, principally in comparative tests. The ox heart after being passed several times through a "mincer" was spread thinly on porcelain and dried with an electric fan and then broken into smaller particles and passed three times through a coffee grinder. Fifty grams of the dried heart muscle were placed in a 500 c.c. Erlen-:meyer flask, and after adding sufficient ether to cover the material it was placed in the ice chest. After 24 hours the yellowish supernatant fluid was poured off and fresh ether was added daily till the supernatant ether remained clear. The ether was then filtered off and the ground muscle spread on paper and dried at room temperature till no ethereal odour could be detected. Twenty grams of this material and 100 c.c. absolute alcohol were mixed and allowed to ex-:tract for 9 days in the ice chest and one day at room temperature.

Tissue extracts were prepared according to the methods described above and then cholesterolized as in the preparation of the antigen used in the system-:atic tests. Comparisons were then made between these and the cholesterolized sheep's heart antigen in use at the time. A cholesterolized ox heart prepar-:ation/

preparation similar to the sheep's heart antigen was also included in the comparative tests and these are shown in the table (vide infra).

Comparison of certain Antigens

Antigens consisting of extracts of sheep's heart and of ox heart were compared with the antigens of Neymann and Gager and Bordet and Ruelens, the latter two having been saturated with cholesterol so as to give a more equal comparison.

The conclusion to be drawn is to the effect that there is little difference between the sheep's heart and ox heart extracts but the Bordet and Ruelens anti-:gen gave two negative results in 2 cases and the Ney-:mann and Gager antigen in one case known to be syph-:ilitic.

Several tests were made and the 6 cases illustrate generally the results.

T A B L E

Doses of Complement:	2	4	6	10	Serum Control 2	Serum Control 4	Antigen
Case I.	0	0	0	t.	j.c.		Sheep's heart extract
	0	0	0	0			Ox heart extract.
	0	0	0	c.			Neymann and Gager.
	0	t.	j.c.				Bordet and Ruelens.
Case II.	0	0	0	t.	c.		Sheep's heart extract
	0	0	t.	a.c.			Ox heart extract.
	0	t.	m.	c.			Neymann and Gager.
	0	m.	c.				Bordet and Ruelens.

Case III/

Doses of Complement:	2	4	6	10	Serum Control 2	Serum Control 4	Antigen
Case III.	0	0	d.	c.	c.		Sheep's heart extract.
	0	0	d.	c.			Ox heart extract.
	0	0	t.	c.			Neymann and Gager.
	0	0	m.	c.			Bordet and Ruelens.
Case IV.	0	t.	m.	c.	c.		Sheep's heart extract.
	0	0	a.c.	c.			Ox heart extract.
	0	0	c.				Neymann and Gager.
	m.	j.c.					Bordet and Ruelens.
Case V.	0	t.	v.m.	c.	c.		Sheep's heart extract.
	0	t.	m.	c.			Ox heart extract.
	0	d.	c.				Neymann and Gager.
	0	d.	c.				Bordet and Ruelens.
Case VI.	0	0	m.	c.	c.		Sheep's heart extract.
	0	0	d.	a.c.			Ox heart extract.
	0	c.					Neymann and Gager.
	v.m.	c.					Bordet and Ruelens.

Antigen Control

Doses of Complement: 2	4	6	
c.			Sheep's heart extract.
c.			Ox heart extract.
c.			Neymann and Gager.
c.			Bordet and Ruelens.

In the above table -

t.= trace.
d.= distinct.
v.m.= very marked.
a.c. = almost complete.
j.c. = just complete.
c. = complete.

Evolution of other tests in the diagnosis of Syphilis

There have been several objections to the Wasser-:mann test such as (1) the technical difficulty in carrying it out, (2) the possibility of fallacious results,/

results, (3) the necessity for a fully equipped labor-
:atory and (4) the need for a thorough training in
serological technique on the part of the worker. It
was but natural then that a more simple test should be
sought for, which would give results equal to the Wasser-
:mann reaction. Moreschi (1905) had observed that
when a soluble protein was mixed with its antiserum pre-
:cipitation occurred and the complement disappeared,
but it is doubtful whether actual complement-fixation
depends on precipitation. Michaelis (1907) noted
the formation of a visible precipitate when the ex-
:tract of a syphilitic liver was brought into contact
with a syphilitic serum. He concluded wrongly that
this observation supported Wassermann's view of the
specific character of the Wassermann test as a true
antigen-antibody reaction. His observation of a pre-
:cipitate may have been a chance one but whether or not,
however, it gave the impetus to those attempts, which
have followed each other down to the present time, to
substitute for the complicated system of the Wassermann
reaction a simple precipitation or Flocculation test.
Soon after this isolated observation by Michaelis,
Porges and Meier (1907-1908) investigated the possi-
:bilities of its practical application, and, strangely
enough, they chose to work not with alcoholic extracts
of organs but with commercial lecithin preparations
from egg yolk, the capacity of which to act as antigen
in/

in the Wassermann reaction had already been demonstrat-:ed. They discontinued the use of lecithin because they found that flocculation occurred with normal serum and because of the instability of lecithin sus-:pensions. Porges, collaborating with Elias, Neu-:bauer and Salomon (1908) used sodium glycocholate, a salt which possesses colloidal properties in solution and which Levaditi and Yamanouchi (1907) had previous-:ly found to function as an antigen in Wassermann's test. Sachs and Rondoni (1908) demonstrated an in-:creased action of lecithin in presence of sodium oleate. Herman and Perutz (1911), taking advantage of the ob-:servations of Browning, Cruickshank and Mackenzie (1910), mixed cholesterol with sodium glycocholate with the object of obtaining a more visible flocculation in the presence of syphilitic serum. These workers showed that this modification was more sensitive than the original antigen of Elias, Neubauer, Porges and Salomon with the sodium glycocholate alone and yielded positive results in certain syphilitic cases where the original method gave no flocculation whatever. There can be little doubt but the flocculation of these lecithin suspensions again drew attention to the study of the underlying mechanism of the Wassermann reaction. Jacobsthal (1910) has shown that, by adding syphilitic serum to alcoholic extracts, (as used in the Wassermann test) precipitates are formed which may be demonstrated by means of dark field illumination, while Bruck and

Hidaka/

Hidaka (1910-1911) obtained similar results by macro-:scopic means by using an extract of liver from cases of congenital syphilis. When the extract was added to syphilitic serum, kept in contact for a prolonged period at a low temperature and centrifugalized, there was a slight precipitate. These observers added gum mastic to the mixtures and by this variation in tech-:nique produced (1) a more bulky precipitate and (2) a result which was more readily observable. The dis-:crepancies, however, with the Wassermann reaction were marked, but their investigations supported the view that the Wassermann reaction depended on the fix-:ation of complement by the formation of an invisible nascent precipitate which occurred in the earliest stage of the interaction between the alcoholic organ extract and the syphilitic patient's serum. Further progress was arrested by the intervention of the War but the increasing number of syphilitic cases to be dealt with under War conditions had the effect of stimulating greatly further effort to elicit a simpler test than the Wassermann reaction. It was not until 1918, however, that Sachs and Georgi, who had an in-:timate knowledge of the comparative values of simple alcoholic extracts and purified lipoids as antigens, used an alcoholic extract of bullock's heart with cholesterol added. Meinicke (1917) was, however, the first/

first to show the reliability of flocculation methods as practical diagnostic aids. He used as antigen an extract of horse's heart in distilled water but did not add cholesterol. The patient's serum was inacti-:vated in the usual way and mixtures of the salt-free antigen and serum were made and kept at 37°C. for 24 hours. This is stage one, and all tubes, whether with normal or luetic serum, gave a definite precipitate with this hypotonic solution. In the second stage, a definite amount of a sodium chloride solution of prev-:iously determined strength was run into each tube, and the tubes were again incubated for an hour. It was now found that in all Wassermann-negative sera the flocculi had disappeared, while in Wassermann-positive flocculation remained unaltered. The salt concentrat-:ion, determined from previous experiment, is that which will in one hour just completely dissolve the flocculi in the normal controls, so that differences can be elicited as between strongly positive, weakly positive, doubtful and negative series. The reaction at first found favour with German serologists but the results obtained have been inferior to those of the Sachs-Georgi method.

The Sachs-Georgi Reaction

As already noted, Sachs and Georgi used a cholest-:erolized antigen. They had previously shown that this/

this addition was not necessary in the case of the Wassermann reaction but was sufficiently advantageous to warrant its almost universal use. It is essential in the Flocculation test, otherwise the flocculi would not be visible. They diluted the cholesterolized alcoholic extract of ox heart with five parts of 0·85 per cent. sodium chloride solution. Serum was in-:activated and diluted 1 : 10. One cubic centimetre of the diluted serum was mixed with 0·5 c.c. of the diluted extract. The usual controls were included. Tubes were either incubated for 24 hours or were kept at 37°C. for 2 hours and then left at room temperature overnight. They recorded three degrees of flocculation-complete, half complete and quarter complete, with neg-:ative cases and controls showing no trace of floccu-:lation.

Further reference will be made later to other serological phenomena that have been studied in the diagnosis of syphilis (See Section D.).

SECTION B.

FLOCCULATION REACTION.

I N T R O D U C T I O N.

SECTION B. FLOCCULATION REACTION

INTRODUCTION

It is now well recognised that the interaction of syphilitic serum and the lipoid "antigen" used in the Wassermann reaction is evidenced not only by a comple-:ment-fixation effect but also under certain conditions by the occurrence of a flocculent precipitate in the mixture of serum and lipoid emulsion. This Flocculat-:ion reaction may be easily visible to the eye in appropriate test-tube experiments and the effect may be regarded, like the complement-fixation reaction, as an indication of some specific interaction between certain constituents of the serum and the lipoid sub-:stances that have been used as antigen in the Wasser-:mann test: whether these complement-fixation and flocculation effects are dependent on the same under-:lying interaction is doubtful but the fact remains that the two phenomena show a definite degree of parall-:elism in the respective tests with syphilitic sera. The question as to whether the Flocculation test can be substituted for the Wassermann reaction in the rout-:ine serum diagnosis of syphilis is becoming one of ever increasing importance. Of course, the technique of the Wassermann test is now firmly founded on many years of experience of the reaction and the technical fallacies that at one time rendered its diagnostic mis-:use so prevalent are well known and carefully guarded against/

against in the methods now in vogue. The test still remains an elaborate one requiring the resources of a well equipped serological laboratory and a degree of training and experience on the part of the laboratory worker both in the carrying out of the method and in the interpretation of the results. There enters into this reaction a biological agent - serum-complement - well recognised by experienced serologists as one of the most complex, unstable and variable of serum princ-:iples. Certain of the difficulties of the Wassermann test are centred in the variability of this reagent. Thus, specimens of complement from different individual animals tend to vary not only in their haemolytic pro-:perties but also in their "deviability" or the degree to which they can be deviated or fixed in a complement-fixation test, such as the Wassermann reaction. In all, five different biological agents enter into the reaction - the lipoid antigen, syphilitic serum, serum complement and the haemolytic system (consisting of red blood corpuscles sensitized with a homologous haem-:olytic antibody). The Flocculation reaction is essentially simple and depends on two only of these re-:agents - the syphilitic serum and the antigen. The technical difficulties and possible fallacies assoc-:iated with the use of the other reagents necessary for the complement-fixation test are eliminated and if it can be shown beyond doubt that the Flocculation reaction is equally reliable and significant with the more/

more elaborate test, its substitution for the Wasser-:mann reaction would be fully justified.

Since the Flocculation phenomenon was first ad-:vocated as a diagnostic method by Sachs and Georgi (1918), various methods of eliciting the reaction and applying it for diagnostic purposes have been intro-:duced and the importance of a quantitative technique has been emphasized. These methods have differed mostly in the type of antigen used and in details of technique, but the actual tests though in some cases described under different designations (e.g. the Sigma reaction of Dreyer and Ward, Kahn reaction, etc.) are essentially the same and dependent on the Flocculation phenomenon referred to above. In 1921 a quantitative method based on the original test of Sachs and Georgi was described by Mackie. The "Antigen" used was a cholesterolized alcoholic extract of sheep's heart. For the test a 1 : 6 emulsion in normal saline prepared by slow admixture was used. This emulsion in bulk exhibits a considerable degree of "turbidity" but is highly stable as regards its physical state, and even after standing for 48 hours shows practically no de-:posit. The reaction was rendered quantitative by testing a fixed quantity of the antigen emulsion with varying dilutions of the serum ranging from 1 : 2 to 1 : 256. The mixtures were placed in narrow agglutin-:ation tubes and incubated for 4 hours at 37°C. and then allowed to stand over night at room temperature. The/

The flocculi that form in the case of a positive re-:action are easily visible to the naked eye and the strength of the reaction was estimated according to the highest dilution in which flocculation occurred. Mackie showed that the proportion of antigen to serum was an important factor in the results and that an ex-:cessive amount of antigen might practically annul the effect. It was also found that the results depended on the physical state of the antigen, the maximum effects occurring with "turbid" emulsions prepared by slow admixture (as contrasted with those obtained by rapid admixture of the alcoholic extract and saline). In an initial comparison between the results of this method and those of the Wassermann reaction it was found that a proportion of sera, positive in the Wasser-:mann reaction, failed to exhibit a positive Flocculat-:ion reaction and the comparison seemed to favour the complement-fixation test. Associated with negative Wassermann reactions, weak flocculation effects were met with, whose diagnostic significance could not be definitely assessed at that time and the question of non-specific reactions was raised. In a later publi-:cation (1923) on the mechanism of the reaction, attent-:ion was drawn to the fact that heating of the serum to 55°C. for half an hour is an important factor in intensifying the flocculation effect and that the max-:imum reaction might only be obtained with sera from recently/

recently withdrawn specimens of blood. Positive sera kept for some days exhibited weaker reactions, marked "zone-effects" and even negative results. Stress was laid on the necessity of testing the sera as soon as possible after removal of the blood. Thus, to secure the optimum results in this test, attention to these details was found to be essential.

As a preliminary to an extensive study of the diagnostic application of the Flocculation test, Ferguson Watson (1925) conducted a further inquiry into certain factors likely to influence the reaction, including that of mechanical shaking of the mixtures as in the method advocated by Kahn (1922-1923). The importance of (1) the particular temperature at which the serum is heated, (2) the testing of the serum as soon as possible after withdrawal of the blood and (3) the relative amounts of antigen and diluted serum, was confirmed and emphasized. Shaking of the mixtures, as in Kahn's method, was found to hasten the reaction and render the flocculation more easily observable to the naked eye. In the subsequent application of the method, mechanical shaking was, therefore, added to the technique (<u>vide infra</u>). The question of the optimum type of antigen was also considered and some comparison was made of various antigens, including those prepared according to the methods of Neymann and Gager (1917), as used by Kahn, and of Bordet and Ruelens/

Ruelens (1919), as used in the "Sigma" reaction. Cholesterolized ox and sheep's heart antigens, prepar-:ed by the methods described later (as recommended for the test by Mackie) were found most uniformly satis-:factory and this type of antigen was used throughout in the systematic tests. The comparative experiments with different antigens will be dealt with later. In the original technique the lowest dilution of serum tested was 1 : 2. The question of including undilut-:ed serum was also considered and it was found that in the case of a positive serum flocculation was generally less with pure serum than when 1 : 2 and 1 : 4 dilut-:ions were used. Having found the optimum conditions for the Flocculation phenomenon, an extensive compari-:son of the Wassermann and Flocculation reactions was carried out with a view to determining their relative value in the serological diagnosis of syphilis (<u>vide Section C. infra</u>).

<u>Preparation of Antigen</u>

The same antigen was used as that employed in the Wassermann reaction and was prepared as follows:-

Heart muscle, freed from fat and vessels, was put through a mincing machine, finely and thoroughly ground with sand and then extracted for 4 days at room temper-:ature with 96 per cent. absolute alcohol (20 grams of heart to 100 c.c. of alcohol); the extract was filter-:ed/

filtered and saturated with cholesterol.

Technique.- Antigen emulsion for the test was made up by slow admixture as a 1 : 6 dilution with normal saline. The patient's serum was heated in the usual way for half an hour. The following dilutions of sera were prepared in small tubes (3" × ½") - 1 : 2, 4, 8, 16, 32 and 64 and to 0·4 c.c. of each, one half volume of antigen suspension was added (i.e. 0·2 c.c.).

Preliminary Investigation

The object of this investigation was to study the various factors that might influence the flocculation phenomenon obtained with syphilitic serum and a lipoid antigen. This question has obviously become an important practical one in view of the application of the Flocculation test in the diagnosis of syphilis as a substitute for the Wassermann reaction. The complexity of the reaction has been specially evidenced by certain observations made by Mackie (1923), and it has been shown that the results depend not only on an active principle in the serum of syphilitic persons but also on an inhibitory substance. An important development in the technique has also been introduced by Kahn, i.e., shaking the mixtures of antigen and serum. Attention has been drawn to the importance of the temperature factor in heating the sera preparatory to the test, as in the case of the Wassermann reaction and/

and to the age of the sera.

The main factors investigated in this research were as follows:-

(1) The effect of heating the serum preparatory to the test at various temperatures, the results being compared with those obtained with unheated serum.

(2) The age of serum, i.e., the time elapsing between the withdrawal of the blood from the patient and the carrying out of the test.

(3) The effect of shaking the mixtures of antigen and sera.

(4) The relative amount of antigen to serum.

(5) The temperature of incubation.

Attention has also been paid in the investigation to various other details in the technique. The technique used in the investigation was that described by Mackie (1921). For control purposes all the sera tested for Flocculation were also submitted to the Wassermann reaction.

The tubes were incubated at 37°C. for 4 hours and then allowed to stand at room temperature over night. Readings were made at 4 hours, 18 to 24 hours and 36 hours. Though in many cases readings were made on negative and control cases up to 48 hours, the results were not always recorded. In some cases the mixtures, after being made in the 3" × ½" tubes, were transferred to narrow agglutination tubes (3" × $\frac{1}{6}$"). The object was to facilitate the observation of the reaction.

(1)/

(1) The effect of heating the serum at various temperatures.

A series of parallel tests were made with unheated serum and serum heated for half an hour at 50°C., 53°C., 54°C., 55°C., 56°C., 57°C. and 58°C. respectively.

Note: The case numbers given in the various tables are only used for convenience to designate the sera whose flocculation effects are selected for illustration.

The following tables exemplify the results:-

TABLE I.

	Serum dilutions					
	1 in 2	1 in 4	1 in 8	1 in 16	1 in 32	1
Case I.						
Serum unheated	++++	++++	+++	++	+	
Serum at 50°C.	-	-	-	-	-	
" " 52°C.	+	+	++	++	+	
" " 53°C.	++	+++	+++	+++	++	
" " 54°C.	+++	+++	+++	++	++	
" " 55°C.	++++	++++	++++	++++	+++	
" " 56°C.	+++	+++	+++	+++	++	
" " 57°C.	++	+++	+++	+++	++	
" " 58°C.	-	-	+	-	-	
Case 2.						
Serum unheated	-	-	-	-	-	
Serum at 50°C.	-	-	-	-	-	
" " 52°C.	-	-	-	-	-	
" " 53°C.	+	++	+	-	-	
" " 54°C.	+++	+++	++	-	-	
" " 55°C.	++++	++++	++++	++	-	
" " 56°C.	++++	++++	+++	+	-	
" " 57°C.	+++	+++	+++	++	-	
" " 58°C.	++	++	+	-	-	

Note: In this and subsequent tables the number of + marks indicates the degree of flocculation.

	1 in 2	1 in 4	1 in 8	1 in 16	1 in 32	1 in 64
Case 3.						
Serum unheated	-	-	-	-	-	-
Serum at 50°C.	+	+	-	-	-	-
" " 52°C.	++	++	-	-	-	-
" " 53°C.	+++	+++	+	-	-	-
" " 54°C.	+++	+++	++	+	-	-
" " 55°C.	++++	++++	++++	+++	++	-
" " 56°C.	++++	++++	++++	+++	++	-
" " 57°C.	+++	+++	+	+	-	-
" " 58°C.	+	-	-	-	-	-

These represent 36 hours' readings. The control containing antigen but no serum showed no trace of flocculation. In Case 1. the blood was withdrawn 12 hours before the experiments; in Case 2., 14 hours and in Case 3., 4 hours. All three cases were diag-:nosed clinically as syphilitic and had not been under treatment. The results in Cases 2. and 3. are spec-:ially noteworthy, in that fresh unheated sera com-:pletely failed to produce a positive reaction, while the heated sera, e.g., 56°C., in both cases yielded a definite reaction. This strongly emphasizes the im-:portance of this factor. In Cases 1. and 2. the optimum temperature was 55°C. while in Case 3. it was 55°C. to 56°C. A similar analysis of sera from a large series of cases showed optimum temperatures vary-:ing from 54°C. to 56°C.

(2) Age of Serum

It was apparent that the age of the serum exerted

a/

a definite influence on the results. Certain positive sera were tested at intervals after withdrawal of the blood up to 10 days and careful precautions were taken to avoid bacterial contamination of the sera. The maximum effects were obtained within 3 days and thereafter the results became progressively weaker and "zone-effects", which have been frequently noted in this reaction, tended to occur. The table of results with sera from cases 1, 2 and 3, already referred to, shows the weakening of the reaction due to age of the sera.

TABLE II.

	Serum dilutions					
	1 in 2	1 in 4	1 in 8	1 in 16	1 in 32	1 in 64
Case 1.						
At 8th day Serum 55°C. for half an hour. . .	+++	+++	+	-	-	-
Case 2.	-	-	-	-	-	-
Case 3.	-	-	-	-	-	-

In the Wassermann reaction, serum 1. showed no lysis with 12 doses of complement - serum 2. no lysis with 8 doses but marked lysis with 12, while serum 3. showed a trace only with 8 doses and distinct lysis with 12.

(3) Shaking

This addition to the technique was originally introduced in the method described by Kahn (1922). A number of comparative tests have been made in which the reaction was carried out in the usual way and also where the tubes containing the mixtures were shaken in a shaking machine for varying periods. (The tubes standing vertically in racks were shaken in a large shaking machine, the racks being fixed in the carrier of the machine). This procedure has proved a valu-:able addition to the technique by (1) hastening the Flocculation reaction and (2) producing larger flakes and so rendering the reaction more easily observed with the naked eye.

It has been found that shaking for 5 minutes be-:fore incubation is sufficiently effective. Thus, a strong positive serum, which showed no flocculation in any dilutions after 4 hours, when tested in the ordin-:ary way yielded flocculation up to a 1 : 32 dilution 5 minutes after the mixture had been made, during which time the tubes were shaken. In many cases, of course, the reaction was not elicited immediately by shaking, but the effect of this procedure was generally to hast-:en the reaction so that positive results could be de-:termined much more quickly than by the ordinary tech-:nique.

TABLES III & IV./

TABLES III & IV.

The following illustrations of the effects of shaking have been selected at random from the series:-

TABLE III

With shaking: 5 minutes

Case	Serum dilutions 1 in 2	1 in 4	1 in 8	1 in 16	1 in 32
1	+++	+++	+++	++	+
2	+++	++	+	-	-
3	++++	++++	+++	++	+
4	+++	++	+++	+++	+++
5	++++	+++	++	+	-
6	++++	++++	+++	++	+
7	++++	++++	+++	++	+
8	++++	++++	+++	++	+
9	++++	++++	++	+	-
Without shaking					
Case					
1	++	++	+	-	-
2	-	-	-	-	-
3	++	++	+	-	-
4	-	-	-	-	-
5	++	+	-	-	-
6	++	++	+	-	-
7	++	++	-	-	-
8	+	+	-	-	-
9	-	-	-	-	-

Readings were taken every hour, but the above re-:presents those taken at the end of 4 hours.

TABLE IV./

TABLE IV.

With shaking: read before incubation at 37°C.

Case	Serum dilutions 1 in 2	1 in 4	1 in 8	1 in 16	1 in 32
1	++	++	-	-	-
2	+++	+++	++	+	-
3	+++	+++	+	-	-
4	-	-	+	++	++
5	++	++	+	-	-
6	+	+	-	-	-
7	+	+	+	+	-

No shaking: read after 1 hour at 37°C.

Case	Serum dilutions 1 in 2	1 in 4	1 in 8	1 in 16	1 in 32
1	-	-	-	-	-
2	-	-	-	-	-
3	-	-	-	-	-
4	-	-	-	-	-
5	-	-	-	-	-
6	-	-	-	-	-
7	-	-	-	-	-

(4) Relative amounts of antigen and serum

The amount of antigen added to the various dilut-:ions of serum was usually one half volume of a 1 : 6 suspension. Thus, with 0·4 c.c. amounts of sera, 0·2 c.c. antigen was added. This was found by comparative tests to be the optimum proportion; with larger amounts of antigen, flocculation was either inhibited or marked "zone effects" occurred, i.e., flocculation only devel-:oped in the higher dilutions. With lesser proport-:ions of antigen the precipitate was so reduced in amount that readings were rendered more difficult.

TABLE V./

TABLE V.

The following table illustrates how the tendency to exhibit a "zone reaction" is marked when a syphilit-:ic serum is 3 days old and over.

Serum over 3 days.	Serum dilutions 1 in 2	1 in 4	1 in 8	1 in 16	1 in 32	1 in 64
1	+	++	+++	++	-	-
2	+	++	+++	+++	++++	-
3	+	++	+++	+++	+++	-
4	++	++	+++	+++	++	+
5	+	++	+++	++++	++++	++++
6	-	-	-	-	+	++
7	-	-	-	+	+++	+++
8	-	+	+	-	-	-
9	+	+	++	++	++	+++
10	-	+	+++	+++	++	+
11	-	-	-	++	+++	++++
12	++	+++	++++	++++	++++	++++
13	-	-	-	+	+++	++
14	++	++	++	+++	+++	+++
15	++	++	+++	++++	++++	+++
16	-	+	++	++++	++++	+++
17	-	-	-	+	+++	+++
18	-	-	-	-	+	++

TABLE VI

Showing the effect of various volumes of antigen.

Case	Antigen	Serum dilutions 1 in 2	1 in 4	1 in 8	1 in 16	1 in 32	1 in 64
1.	2 Vols.	-	-	-	-	++	+++
	1 Vol.	-	-	-	++	+++	++++
	½ Vol.	++++	++++	++	-	-	-
	¼ Vol.	+++	+++	+	-	-	-
	⅛ Vol.	++	++	+	+	-	-
2.	2 Vols.	-	-	-	-	-	-
	1 Vol.	-	-	-	-	-	-
	½ Vol.	+++	+++	++	+	-	-
	¼ Vol.	+++	++	+	+	-	-
	⅛ Vol.	+	+	+	++	-	-

3/

Case	Antigen	Serum dilutions 1 in 2	1 in 4	1 in 8	1 in 16	1 in 32	1 in 64
3.	2 Vols.	++++	++++	++++	+++	++	-
	1 Vol.	++++	++++	++++	++++	+++	++
	½ Vol.	++++	++++	++++	++++	++++	+++
	¼ Vol.	+++	+++	+++	+++	+++	+++
	⅛ Vol.	++	++	++	++	++	++
4.	2 Vols.	+	++	+	-	-	-
	1 Vol.	++++	++++	+++	++	+	-
	½ Vol.	++++	++++	++++	++++	++	+
	¼ Vol.	+++	+++	+++	+++	+++	++
	⅛ Vol.	++	++	++	++	++	++

It will be observed that the optimum reaction is with ½ volume of antigen in Cases 2, 3 and 4. Case 1. gives a remarkable result as there is occurrence of flocculation in high dilution only when an excess of antigen is used.

(5) Temperature of Incubation

The usual technique has been to incubate the tubes for 4 hours at 37°C. The reaction may, however, devel-:op at room temperature though generally slower even in strong positive cases. In certain instances where the test was carried out with positive sera without shaking, no flocculation occurred at room temperature, whereas a marked effect was elicited by incubation. Incubat-:ion at 57°C. yielded very weak flocculation effects as compared with those at 37°C.

(6) Rate of development of the reaction

It is to be specially noted that in the absence of shaking the reaction may be slow in developing. The time the reaction takes is exceedingly variable; in some cases the results are easily read even after one/

one hour, but in other cases with strong Wassermann reactions no effects were elicited until 24 hours had elapsed and in a few exceptional instances a positive result was only observable after 36 hours.

TABLE VII.

The following table illustrates the approximate rate of development of the reaction. In these cases the mixtures were not subjected to mechanical shaking as described above.

	Serum dilutions						
Case	1 in 2	1 in 4	1 in 8	1 in 16	1 in 32	1 in 64	Read-ings. Hours
1.	+	-	-	-	-	-	4
	++	-	-	-	-	-	12
	++	+	-	-	-	-	18
	+++	++	+	-	-	-	24
	+++	+++	++	+	-	-	36
2.	-	-	-	-	-	-	4
	+	+	-	-	-	-	12
	+++	++	+	-	-	-	18
	+++	+++	++	++	+	-	24
	++++	++++	++++	+++	+	-	36
3.	++	+	-	-	-	-	4
	+++	+++	+	-	-	-	12
	++++	++++	++	+	-	-	18
	++++	++++	+++	+	-	-	24
	++++	++++	++++	++	+	+	36
4.	-	-	-	-	-	-	4
	+	-	-	-	-	-	12
	++	+	-	-	-	-	18
	+++	+++	+	-	-	-	24
	++++	++++	++	++	+	+	36
	++++	++++	++++	++++	+++	+++	48

TABLE VIII./

TABLE VIII.

This table illustrates the rate of development of the reaction after shaking.

Case	Serum dilutions 1 in 2	1 in 4	1 in 8	1 in 16	1 in 32	1 in 64	Readings. Hours
1.	++++	++++	+++	++	-	-	1
	++++	++++	+++	++	-	-	2
	++++	++++	+++	++	+	-	3
	++++	++++	+++	++	+	-	4
	++++	++++	++++	++	+	-	12
	++++	++++	++++	++	+	-	24
	++++	++++	++++	+++	+	+	36
2.	+++	+++	+	-	-	-	1
	+++	+++	++	-	-	-	2
	+++	+++	++	+	-	-	3
	++++	+++	++	+	-	-	4
	++++	++++	+++	+	-	-	12
	++++	++++	+++	+	-	-	24
	++++	++++	+++	+	-	-	36
3.	+++	+	-	-	-	-	1
	+++	+	-	-	-	-	2
	+++	++	-	-	-	-	3
	+++	++	-	-	-	-	4
	+++	++	+	-	-	-	12
	+++	++	+	-	-	-	24
	++++	+++	++	-	-	-	36
4.	+	-	-	-	-	-	1
	++	++	++	+	-	-	2
	+++	+++	++	+	-	-	3
	++++	++++	+++	++	-	-	4
	++++	++++	+++	++	-	-	12
	++++	++++	+++	++	+	-	18
5.	++	+	-	-	-	-	1
	++	++	+	-	-	-	2
	+++	+++	++	+	-	-	3
	++++	++++	+++	++	+	-	4
	++++	++++	++++	+++	+++	++	12
	++++	++++	++++	++++	++++	++++	18

(7) Various details in technique.

Size of tubes.- For making the dilutions of serum and the mixture with antigen, wide tubes, such as those used in the Wassermann reaction (3" × ½") were used. If the mixtures are ultimately transferred to narrow agglutination tubes (3" × $\frac{1}{6}$") the readings are considerably facilitated. Shaking is more eff-:ectively carried out in the wide than in the narrow tubes. It is therefore advisable to shake the mix-:tures before transfer to the agglutination tubes.

Naked-eye and lens readings.- Reference has already been made to the effect of shaking in render-:ing the flocculi larger and more easily noted with the naked eye. Thus shaking assists materially in the ultimate reading. Some observers use a dark background in reading the results but no difficulty has been encountered in making the readings with the naked eye, especially when the tubes were shaken. The use of a lens is of value in detecting weak effects.

Rotation of tubes.- When agglutination tubes were used, rapid rotation between the palms of the hands before reading results evenly distributed the flocculi in the tubes and the degree of flocculation could at once be determined.

THE PRINCIPAL INVESTIGATION

In this part of the research 2,821 tests were carried out. Comparative results are dealt with in Section C. but the alcohol tests and the animal ex-:periments are not included.

The influence of mechanical shaking was further investigated and the conclusion arrived at was that shaking for a period of 5 minutes before incubation gave the best results - a conclusion which confirmed a previous opinion.

TABLE IX.

This table illustrates differences between un-:heated sera, sera heated to 55°C. for half an hour and left at room temperature, and sera at 55°C. for half an hour and then incubated for 4 hours at 37°C. (with and without shaking).

Case	Serum dilutions 1 in 2	1 in 4	1 in 8	1 in 16	1 in 32	1 in 64	Tempera-:ture	Shaking Minutes
1. Unheated	-	-	-	-	-	-	Incubator	5
At 55°C. ½ hr.	++++	++++	+++	++	+	-	Incubator	None
" 55°C. ½ hr.	++++	++++	+++	++	+	-	Room	5
" 55°C. ½ hr.	++++	++++	++++	+++	+	+	Incubator	5
2. Unheated	+++	+++	++	+	-	-	Incubator	5
At 55°C. ½ hr.	++++	++++	++++	+++	+	-	Incubator	None
" 55°C. ½ hr.	++++	++++	++++	+	-	-	Room	5
" 55°C. ½ hr.	++++	++++	++++	+++	+	-	Incubator	5
3. Unheated	+	+	-	-	-	-	Incubator	5
At 55°C. ½ hr.	++	+	+	-	-	-	Incubator	None
" 55°C. ½ hr.	+	+	-	-	-	-	Room	5
" 55°C. ½ hr.	++++	++++	+++	+	-	-	Incubator	5
4. Unheated	+	-	-	-	-	-	Incubator	5
At 55°C. ½ hr.	++	+	-	-	-	-	Incubator	None
" 55°C. ½ hr.	-	-	-	-	-	-	Room	5
" 55°C. ½ hr.	++++	++++	++++	+++	+	-	Incubator	5

TABLE X.

This table is intended to show that shaking for 5 minutes is quite as effective as compared with more prolonged shaking.

Case	Serum dilutions 1 in 2	1 in 4	1 in 8	1 in 16	1 in 32	Shaking. Minutes.
1.	++++	++++	+++	++	-	5
	++++	++++	+++	++	-	10
	++++	++++	+++	+	-	20
	+++	+++	++	+	-	25
2.	++++	++++	+++	++	+	5
	++++	++++	+++	++	+	10
	++++	++++	+++	++	+	20
	++++	++++	+++	++	+	25
3.	++++	++++	+++	+	-	5
	++++	++++	++	+	-	10
	++++	+++	+	-	-	20
	++++	++++	++	+	-	25
4.	++	++	-	-	-	5
	++	++	-	-	-	10
	++	++	-	-	-	20
	++	+	-	-	-	25
5.	++++	++++	+++	++	-	5
	+++	+++	+++	+	-	10
	++++	++++	+++	++	-	20
	+++	+++	++	+	-	25
6.	+++	++	-	-	-	5
	+++	++	-	-	-	10
	+++	++	+	-	-	20
	-	-	-	++	-	25
7.	+++	+++	++	+	-	5
	+	+++	++	++	-	10
	++	++	+	-	-	20
	-	+	+	+	-	25
8.	-	-	-	-	-	5
	-	-	-	-	-	10
	-	-	-	-	-	20
	-	-	-	-	-	25

Readings were made after shaking and at 1, 2, 3, 4, 12, 18, 24, and 36 hours. Prolonged shaking weakens the effect. Mechanical shaking for a pro-:longed period does not elicit false positive results with negative sera.

TABLE XI/

TABLE XI.

This table refers to certain known positive cases (which were tested unheated and also heated at 55°C. for half an hour), shaken for 5 minutes and left at room temperature instead of being incubated at 37°C.

	Serum dilutions						
Case	1 in 2	1 in 4	1 in 8	1 in 16	1 in 32	1 in 64	Serum
1.	-	-	-	-	-	-	Unheated
	++++	++++	++	+	-	-	55°C.
2.	-	-	-	-	-	-	Unheated
	+++	+	-	-	-	-	55°C.
3.	++	++	-	-	-	-	Unheated
	++	+	-	-	-	-	55°C.
4.	+++	++	-	-	-	-	Unheated
	++++	+++	++	+	-	-	55°C.
5.	-	-	-	-	-	-	Unheated
	-	-	-	-	-	-	55°C.

It should be specially noted that 3 of the unheated and 1 of the heated cases failed to react at room temperature though the time of year was June 21.

The question of including undiluted serum has already been referred to on page 32 and the general results obtained are illustrated in the following table.

TABLE XII.

Comparison between undiluted and diluted serum.

Case	Serum dilutions: Undiluted	1 in 2	1 in 4	1 in 8	1 in 16	1 in 32	Readings Hours.
1.	-	+	+	-	-	-	4
	+	+	+	-	-	-	18
	+	++	++	-	-	-	24
	++	+++	+++	++	+	-	36
2.	+	+	+	+	-	-	4
	++	++	++	+	-	-	18
	++	++	++	+	+	-	24
	+++	+++	+++	+++	++	+	36
3.	-	++	++	++	++	-	4
	-	+++	+++	+++	++	+	18
	+	+++	+++	+++	++	+	24
	++	+++	+++	+++	++	+	36
4.	-	+	+	-	-	-	4
	-	++	+	-	-	-	18
	+	++	++	+	-	-	24
	+	+++	+++	++	+	+	36
5.	-	+	+	-	-	-	4
	-	++	+	-	-	-	18
	+	+++	+	+	-	-	24
	+	+++	++	+	-	-	36
6.	-	+	+	-	-	-	4
	+	++	+	-	-	-	18
	+	+++	+	+	-	-	24
	+	+++	++	+	-	-	36
7.	+	+	-	-	-	-	4
	+	++	+	-	-	-	18
	++	+++	++	+	-	-	24
	++	+++	+++	++	+	-	36
8.	++	+	-	-	-	-	4
	++	++	+	-	-	-	18
	+++	+++	++	++	-	-	24
	+++	++++	++++	+++	++	+	36

TABLE XIII/

TABLE XIII.

This table shows (a) comparisons between sheep's heart, ox heart, Neymann and Gager and Bordet and Ruelens Antigens and (b) comparative results between 6 sheep's heart antigens which were made at the same time and by identical methods. (See Section A. p.19.)

(a)

Case	Serum dilutions. 1 in 2	1 in 4	1 in 8	1 in 16	1 in 32	Antigen.
1.	++++	++++	++++	++	+	Sheep's heart extract
	++++	++++	+++	++	+	Ox heart extract.
	++++	++	+	+	-	Neymann and Gager.
	+++	++	++	-	-	Bordet and Ruelens.
2.	++++	+++	++	+	-	Sheep's heart extract
	++++	+++	++	+	-	Ox heart extract.
	+++	+++	++	+	-	Neymann and Gager.
	++	++	+	-	-	Bordet and Ruelens.
3.	++++	++++	++++	++	+	Sheep's heart extract
	++++	++++	++++	++	-	Ox heart extract.
	++++	+++	++	+	-	Neymann and Gager.
	+++	++	++	-	-	Bordet and Ruelens.
4.	+++	++	++	+	+	Sheep's heart extract
	+++	++	++	+	+	Ox heart extract.
	+++	++	+	-	-	Neymann and Gager.
	++	++	+	-	-	Bordet and Ruelens.
5.	+++	++	+	+	-	Sheep's heart extract
	+++	++	+	+	-	Ox heart extract.
	+++	++	+	-	-	Neymann and Gager.
	++	++	+	-	-	Bordet and Ruelens.
6.	++++	+++	+++	+	-	Sheep's heart extract
	++++	+++	++	+	-	Ox heart extract.
	+++	+++	+	-	-	Neymann and Gager.
	+++	++	+	-	-	Bordet and Ruelens.

All readings made after 36 hours.

(b)/

(b)

Case	Serum dilutions. 1 in 2	1 in 4	1 in 8	1 in 16	1 in 32	Antigen.
1.	++	+	-	-	-	Sheep's heart extract VII
	++	+	-	-	-	" " " VIII
	++	+	-	-	-	" " " IX.
	++	+	-	-	-	" " " X
	++	+	-	-	-	" " " XI
	++	+	-	-	-	" " " XII
2.	++++	++	+	-	-	Sheep's heart extract VII
	++++	++	+	-	-	" " " VIII
	++++	++	+	-	-	" " " IX
	++++	+++	+	-	-	" " " X
	++++	+++	+	-	-	" " " XI
	++++	+++	+	-	-	" " " XII
3.	+++	++	+	-	-	Sheep's heart extract VII
	++++	++	+	-	-	" " " VIII
	++++	++	+	-	-	" " " IX
	++++	++	+	-	-	" " " X
	+++	++	+	-	-	" " " XI
	+++	++	+	-	-	" " " XII
4.	-	-	-	-	-	Sheep's heart extract VII
	-	-	-	-	-	" " " VIII
	-	-	-	-	-	" " " IX
	-	-	-	-	-	" " " X
	-	-	-	-	-	" " " XI
	-	-	-	-	-	" " " XII
5.	++++	++++	+++	++	-	Sheep's heart extract VII
	++++	++++	+++	+	-	" " " VIII
	++++	++++	+++	++	-	" " " IX
	++++	++++	+++	++	-	" " " X
	++++	++++	+++	+	-	" " " XI
	++++	++++	+++	+	-	" " " XII

Previous experiments had shown that comparison between various antigens, made at different times, gave variable results, in spite of the fact that as each antigen was made careful titration with the prev-:ious stock antigen had been carried out. This is illustrated in Table XIV.

TABLE XIV/

TABLE XIV.

Case	Serum dilutions. 1 in 2	1 in 4	1 in 8	1 in 16	1 in 32	Antigen.	
1.	++	++	+	+	+	Sheep's heart extract	II
	+++	++	+	-	-	" " "	III
	++	+	-	-	-	" " "	IV
	++	++	+	-	-	" " "	V
	++	+	-	-	-	" " "	VI
	++	++	++	+	-	Ox's heart extract.	
2.	+++	+++	++	++	+	Sheep's heart extract	II
	++	++	++	++	+	" " "	III
	+	+	+	+	-	" " "	IV
	+++	+++	+++	++	++	" " "	V
	+	-	-	-	-	" " "	VI
	++	++	++	+	-	Ox's heart extract.	
3.	++	+	-	-	-	Sheep's heart extract	II
	++	+	+	-	-	" " "	III
	-	-	-	-	-	" " "	IV
	-	-	-	-	-	" " "	V
	-	-	-	-	-	" " "	VI
	++	+	+	-	-	Ox's heart extract.	
4.	++	+	-	-	-	Sheep's heart extract	II
	++	++	+	-	-	" " "	III
	-	-	-	-	-	" " "	IV
	++	-	-	-	-	" " "	V
	++	+	+	-	-	" " "	VI
	++	+	-	-	-	Ox's heart extract.	
5.	+++	+++	++	++	+	Sheep's heart extract	II
	++	++	++	+	-	" " "	III
	+	+	+	+	+	" " "	IV
	+++	+++	+++	++	++	" " "	V
	+	-	-	-	-	" " "	VI
	++	++	++	+	+	Ox's heart extract.	
6.	+++	++	++	+	+	Sheep's heart extract	II
	++	+	+	-	-	" " "	III
	+++	+++	++	+	-	" " "	IV
	+++	++	+	-	-	" " "	V
	+++	++	++	+	+	" " "	VI
	+++	++	++	+	-	Ox's heart extract.	

The/

The observation by Dunlop (1924) to the effect that the same serum tested on several successive days gives variable results, was not confirmed. Some sera were tested as often as 7 times and the result never varied, except for progressive weakening and the tendency to zone reactions.

TABLE XV.

Case	Serum dilutions. 1 in 2	1 in 4	1 in 8	1 in 16	1 in 32	1 in 64	Age of serum. Days.
1.	++++	++++	+++	+	-	-	1
	++++	++++	+++	+	-	-	2
	++++	++++	+++	+	-	-	3
	++++	++++	+++	+	-	-	4
2.	++++	++++	++++	++++	+++	+	1
	++++	++++	++++	++++	+++	+	2
	++++	++++	++++	++++	+++	+	3
	++++	++++	++++	++++	+++	-	4
	++++	++++	+++	+	-	-	5
	+++	+++	+	+	-	-	6
	+++	+++	++	-	-	-	7
	+	+++	++	-	-	-	8
3.	+++	+++	+++	++	+	-	1
	+++	+++	+++	++	+	-	2
	+++	+++	+++	++	+	-	3
	+++	+++	+++	++	+	-	4

Cases 1. and 3. show no variation when tested on 4 successive days while case 2. shows progressive weakening of the reaction after the third day from withdrawal of the specimen and a zone effect is shown on the eighth day.

CONCLUSIONS/

CONCLUSIONS

The practical conclusions to be drawn from these observations are as follows:-

(1) The sera should be heated at 54°C. to 56°C. for half an hour and unheated serum should not be used for the test.

(2) After the mixtures of diluted serum and antigen are made, the tubes should be shaken in a shak-:ing machine for 5 minutes by the method described.

(3) Readings should be made after shaking and at intervals, if possible, up to 24 hours. It is also advisable to make a second reading after 36 hours.

(4) Shaking for a period of 5 minutes is quite as effective as shaking for longer periods.

(5) Where the serum for some reason cannot be tested until several days have elapsed after with-:drawal of the blood, negative results are of no significance and the Wassermann reaction must be used.

(6) Better results are obtained with diluted than with undiluted serum.

(7) The Flocculation reaction by the technique indicated is easily carried out as compared with the Wassermann reaction and is applicable even in the absence of complete laboratory resources. Thus, more use might be made of it for diagnostic purposes in the smaller hospitals which are isolated from large centres.

(8)/

(8) With a satisfactory technique the results are, in untreated cases at least, quite as reliable as those of the Wassermann reaction which requires specially skilled work and full laboratory resources.

SECTION C.

Comparison of Wassermann and Flocculation Tests.

INTRODUCTION

SECTION C.

Comparison of Wassermann and Flocculation Tests.

INTRODUCTION

A considerable amount of research work has been done on the more direct and obvious properties of the colloidal lyphobe sols which we designate "antigens", that is to say, the antigens used in the Wassermann and Flocculation tests; and the advantages of using the identical antigen, e.g., the cholesterolized sheep's heart extract, as recommended by Mackie (1920) for both reactions are probably apparent, more especially because the early work of Sachs and Georgi (1918) showed a close degree of parallelism. Dreyer (1921) thought that most probably the thermostable substance in the serum caused the flocculation in the Sigma and Sachs-Georgi tests, and that the thermolabile substance was responsible for the inhibition of flocculation sometimes seen in the higher concentrations and concluded by asserting that it was probably the occurrence of inhibition which interfered with the Sachs-Georgi reaction. Mackie (1923), in an investigation on the serum constituents responsible for the Sachs-Georgi and Wassermann reactions, showed that the complement-fixing principle of the serum was found mainly in the carbonic-acid-insoluble globulin fraction and that the flocculating principle resided mainly/

mainly in the carbonic-acid-soluble fraction of the serum. Kapsenberg (1921) has also shown by the ammonium sulphate method that the Wassermann reaction depends entirely on the globulin of the serum. Wassermann, Neisser and Bruck (1906) assumed that the spirochaetes acted as a true antigen, but later work demonstrated that the lecithin which was extracted at the same time as the spironemata played an important part. Browning and Mackenzie (1910) showed the value of adding cholesterol to the antigen used in the Wassermann reaction and Dreyer and Ward (1921) improved the antigen of Bordet and Ruelens (1919) by showing that the addition of cholesterol though un-:necessary in the Wassermann reaction was essential for the Flocculation test. Taniguchi (1920) has drawn attention to the fact that in human sera there is no constant relationship between syphilitic infect-:ion and the presence of sheep haemolysin - the latter, therefore, constitutes a source of fallacy in the Wassermann reaction when alcoholic extracts of heterogenetic antigens, such as guinea pig's or horse's heart are employed. Since it has been shown that the reacting substances in the Wassermann and Flocculation reactions are not entirely dependent on the same serum constituents it, therefore, follows that one or other of the reactions may sometimes fail to give a positive reaction in cases which are clini-:cally syphilitic or in cases which are under treat-:ment. Ferguson Watson (1926) has drawn attention

to/

to cases under treatment which give negative results and has emphasized the necessity of testing all treated cases once a month after treatment has been suspended. Even under optimum conditions the Wassermann reaction may fail, and, as pointed out by Mackie and Rowland (1920), this, as is well known, represents a practical limitation which detracts con-:siderably from the diagnostic application of the test. In a reaction which depends on five differ-:ent biological reagents - apart from variability of these constituents - there is more complexity than in a test which depends on serum and antigen alone. It, therefore, seemed of importance to carry out an extensive investigation with a view to determining the relative diagnostic value of the two reactions; and after having ascertained the factors which were responsible for ensuring optimum conditions in the Flocculation test the present investigation was carried out. Of the 3,283 tests made, a number represent tests with animal sera, repeated tests with the same sera and controls. Excluding those which are dealt with in other sections, all sera known to be "over age", controls and repeats, the number of different cases dealt with in this section number 1,211, and a paper dealing with "The substitution of the Flocculation test for the Wassermann reaction" has been published in the Edinburgh Medical Journal (1927).

Comparative results of other workers

As already stated, the literature on the subject has become voluminous and only a proportion of the comparative work of others can be dealt with, but special care has been taken to make it as represent-:ative as possible.

Sachs and Georgi (1918) using the same antigen for the Sachs-Georgi and Wassermann reactions claimed that their results ran parallel not only with both tests but with clinical findings, and that the Wasser-:mann and flocculation phenomena indicate the same interaction between the serum and lipoid antigen. In a review of cases collected from the literature (1920) they found that the two tests agreed in 92·4 per cent.

Wolffenstein (1919) observed a large proportion of non-specific results with the Sachs-Georgi test. In a series of 230 non-syphilitic sera he obtained 22 positive results and concluded that the new test was of less practical value than the Wassermann re-:action.

Bok (1920) declared that the Flocculation test was more reliable than the Wassermann reaction and that it might enable one to obtain a deeper insight into the chemistry of syphilis than had hitherto been possible.

Dekenga and Platenga (1920) concluded that in cases of syphilis with a strong Wassermann reaction, the/

the Flocculation test was also positive, but where the Wassermann reaction was weakly positive the Flocculation test was often negative and that the Sachs-Georgi test becomes negative more rapidly as a result of treatment.

Pincherle (1920) found that the Flocculation test was specific for syphilis and though less sensi-:tive in cases of incipient disease of the nervous system was useful in clearing up doubtful cases. Hajos and Molnar (1920) concluded that the Sachs-Georgi test was equal to the Wassermann reaction as regards specificity.

Brownlie (1921) in an examination of 500 cases obtained a greater percentage of positive cases by the Sachs-Georgi method than by the complement-fixat-:ion test in cases of primary, secondary and tertiary syphilis.

Mackie (1921) examined 226 cases and found agree-:ment in 93·3 per cent. and formed the opinion that the Flocculation test, even with the most appropriate technique, was less sensitive than the Wassermann re-:action when carried out under optimum conditions. In a later communication (1923) he has drawn attent-:ion to certain factors which elicit optimum results with the Sachs-Georgi test and his findings were not only confirmed but emphasized by Ferguson Watson (1925).

Gutfeld (1921) examined the sera from 1,500 cases and/

and found agreement with the Wassermann reaction in 94·9 per cent. He does not think there is anything to recommend a second reading of the Flocculation test after 24 hours.

Hecht (1921) obtained results which "were in fair agreement" with the Wassermann reaction.

Kahn (1921) described a method which he claimed possessed advantages over the Sachs-Georgi and Sigma methods because of (1) greater stability of the dilut-:ed antigen, (2) spontaneous precipitation with strong positive sera, (3) by reduction of the incubation period to 3 hours and (4) by reading the results with the naked eye. At that time he used the antigen of Neymann and Gager (1917) made from guinea pig's heart and recorded agreement in 98 per cent. In 1925, after testing 101,000 sera, Kahn found that there was remarkable agreement between the Flocculation test and clinical findings and says the test is a reliable method for laboratory diagnosis of syphilis and it can be used alone with safety.

Webb (1922) found agreement with the two tests in 74 per cent., and of the remaining 26 per cent. 19 per cent. favoured the Wassermann test and 7 per cent. the Flocculation, and concluded that the latter was not a reliable index of cure.

Rice (1922) found agreement in 94·4 per cent. but considered the Sachs-Georgi test less sensitive with the/

the sera of patients who were under treatment though quite as reliable in untreated primary, secondary and tertiary syphilis.

Collier (1922) examined 569 cases and concluded that the Flocculation test was more reliable and more delicate than the Wassermann reaction.

Craig and Williams (1922) obtained agreement in 85·5 per cent. and concluded that the disagreements were so radical that the Sachs-Georgi reaction alone should not be relied on and though interesting from a scientific point of view was inferior to the Wasser-:mann reaction for practical purposes.

Taylor (1922) examined 700 sera and found agree-:ment in 92·2 per cent.

Parthasarathy and Barratt (1922) in an examinat-:ion of 265 sera found agreement in 242 and disagree-:ment in 23.

Rook (1922) thinks that the Sigma reaction of Dreyer and Ward is as good as - or even better than - the Wassermann reaction.

Keim and Wile (1922) believe that the Flocculat-:ion test, because of its simplicity, rapidity of reading and reduction of sources of error, possesses advantages over the Wassermann reaction.

Ide and Smith (1922) examined 2,165 sera and be-:lieve the Flocculation test is useful as a check on the Wassermann reaction.

Manheims/

Manheims (1923) in his examination of 500 sera found the Flocculation test more difficult to read but it did not give false positives.

Kolmer (1923), in an analysis of 24,000 cases taken from the literature, found that there was agree-:ment in comparative tests in from 80 to 95 per cent. with a general average of about 89 to 90 per cent. About 8 per cent. of the differences were due to positive complement-fixation and negative Flocculation reactions and 3 per cent. to negative complement-fixation and positive Flocculation reactions.

Young (1923) examined 23,000 sera and found agreement in 93 per cent. while Dawson and Smith(1923) obtained 94·6 per cent. from their 527 cases.

Rockstraw and Bent (1924) made 1,002 tests and found the Kahn test more sensitive than the Sachs-Georgi and agreeing with the Wassermann reaction (cholesterolized antigen) in 96·2 per cent. In un-:treated cases there was agreement in 98·9 per cent. but in treated it fell to 86·5 per cent.

Demanche and Gueriot (1924) found the Vernes test inferior to the Wassermann reaction - that it was frequently negative in the primary stage, in latent and in treated syphilis where the Wassermann reaction was positive.

Osmond/

Osmond and McClean (1924) tested 500 sera and had agreement in 389. Of the 111 not in agreement, 24 favoured the Wassermann reaction and 87 the Kahn test.

Altounyan (1924) examined 227 cases and there was agreement in all but 16 and concluded that the Flocculation test was as good as the Wassermann re-:action but required greater care in reading.

McGlumphy (1924) had agreement in 95 per cent. in his series and says that, if glycerol is added in amount sufficient to have present in each Flocculat-:ion reaction 0·05 to 0·1 c.c., this will facilitate the discovery of flocculation by producing a clear back-:ground and the addition of 3 per cent. sodium chlor-:ide facilitates the reaction. He concluded that the test is not quite so sensitive as the Wassermann reaction but it does not err on the side of false positives.

Cohen and Haythorn (1924) made 1,400 tests and disagreement was noted in 3·9 per cent. These dis-:cordant results were all from patients under treat-:ment and in the later months of pregnancy.

Ebert and Mitchell (1924) concluded that the Flocculation test was specific for syphilis, agreeing with the Wassermann reaction in untreated secondary and congenital syphilis but becoming positive later than the Wassermann reaction in untreated primary syphilis. It is less sensitive in treated cases and unsatisfactory/

unsatisfactory in syphilis of the central nervous system.

Schuler (1925), in an examination of over 5,000 cases, concluded that the Bordet-Wassermann method gave the greatest number of positive results in cases of certain syphilis and considered the Sachs-Georgi precipitation test the most favourable of all the Flocculation tests, being the best method of control for the Wassermann reaction.

Keim and Kahn (1925) in an article in which they discuss the specificity of the test examined 2,500 cases clinically free from syphilis and 2,493 gave a negative Flocculation test; a + reaction was obtain-:ed in 4 cases, a ++ in 2 cases and a +++ in one case.

Kelly (1925) observed a remarkable concordance of results in comparative tests (95·45). In the 5 cases which diverged, 4 were known to have syphilis but the author offers no explanation for the diverg-:ence.

Owen (1926) in an examination of 1,600 sera by the Wassermann and Flocculation methods obtained agreement in 93·8 per cent. and noted that the diverg-:ence between the two tests lies almost wholly among the treated cases. He concluded that the Flocculat-:ion test formed a valuable check to the Wassermann reaction and could "pick up" a certain small percent-:age of positive results which were missed by the older method.

Webb/

Webb (1926), in emphasizing the fact that the antigen extract mixture should be as turbid as poss-:ible, stated that flocculation was easily distin-:guished by the naked eye and great confidence could be placed on a positive reaction.

It is of interest to note that for some time the laboratory of the Michigan Department of Health has ceased to use the Wassermann test on routine specimens for the diagnosis of syphilis, only the Flocculation test being reported on. The Michigan Commissioner of Health (1925), in giving reasons for the change in procedure, avers that the simplicity and accuracy of the Flocculation test in the hands of a well trained personnel make it a more dependable test than that of the Wassermann, as ordinarily carried out by diagnost-:ic laboratories.

The following table gives a synopsis of the per-:centage results of various Authors, arranged accord-:ing to percentage and with the date of publication.

TABLE

NAME	YEAR	PERCENTAGE AGREEMENT
Moody	1923	98·8
Perry and Lambkin	1922	98·5
d'Aunoy	1921	98·07
Kahn	1921	98·0
Clark	1926	97·5
Burtscher	1926	96·6
Chang	1922	96·3
Rockstraw and Bent	1924	96·2
Cohen and Haythorn	1924	96·1

Giordano/

NAME	YEAR	PERCENTAGE AGREEMENT
Giordano	1926	96·0
Parthasarathy and Barratt.	1922	95·9
Herrold	1922	95·5
Kelly	1925	95·45
Aziazzi and Pico	1920	95·43
McGlumphy	1924	95·0
Gutfeld	1921	94·9
Dawson and Smith	1923	94·6
Rice	1924	94·4
Feinblatt	1922	93·8
Owen	1926	93·8
Marcora	1920	93·6
Dreyer	1921	93·5
Zeissler	1922	93·4
Mackie	1921	93·3
Parker and Haig	1921	93·07
Young	1923	93·0
Altounyan	1924	92·8
Sachs and Georgi	1920	92·44
Taylor	1922	92·2
Levinson and Petersen	1921	92·0
Parthasarathy and Barratt.	1922	91·4
Taniguchi	1921	91·0
Baumgartel	1920	90·0
Kolmer	1923	90·0
McGlumphy	1924	90·0
Mustard	1921	90·0
Taniguchi and Yochinare	1921	90·0
Luza	1920	89·3
Levinson	1921	88·8
Dulaney	1923	87·7
Bigger	1924	86·9
Craig and Williams	1922	85·5
Misserschmidt	1920	85·1
Strumia	1923	83·4
Kumer	1920	81·0
Cornwall	1922	81·0
Kirschner and Segall	1920	80·0
Vitringa	1922	80·0
Osmond and McClean	1924	77·8
Webb	1922	74·0

METHODS

The preparation of the antigen was made according to Mackie's method (1920) and, as already stated, the same/

same antigen was used for the Wassermann and Floccu-:lation tests. Technique has already been described.

Criteria of positive reactions.

Wassermann reaction.- The system advocated by Browning and Kennaway (1919) was generally adopted.

Sera which allowed of complete lysis with the same minimal amount of complement as the known nega-:tive control serum were classified, of course, as negative; sera which did not permit complete lysis with less than twice the amount of complement requir-:ed for the negative control serum - allowance being made for any inhibition by the serum alone - were classified as positive; in those tests in which a difference between the serum in question and the nega-:tive control was elicited, though less than that accepted as the criterion of a positive reaction, were classified as doubtful; these types of reaction are illustrated in the tables.

Flocculation test.- Any degree of flocculation noted after 36 hours was recorded and provisionally stated as a positive reaction. In certain cases before shaking had been systematically carried out (vide supra) results after 48 hours were also included.

It was found that normal sera, even in the lowest dilution, yielded no degree whatever of flocculation or precipitate even after 48 hours.

The diagnostic significance of the results will be/

be discussed later.

Results of the comparative tests.

The number of cases examined was 1,211 and it was arranged that all laboratory tests should be completed before any reference was made to clinical data or to the history of the case. One thousand, one hundred and seventy-three (i.e. 96·86 per cent.) of the results were in agreement while 38 (i.e. 3·13 per cent.) differed. Five were classified as positive in the Wassermann reaction and negative in the Flocculation test, 21 as positive in the Flocculation test and negative in the Wassermann reaction, while 12, classified as doubtful results in the Wassermann reaction, were positive in the Flocculation test. It is impossible here to tabulate in detail the results of all the tests carried out. Table I. shows the classification of results. Table II. is intended to illustrate briefly the general types of unequivocal results met with in both tests, sera 1 to 15 being positive, 16 to 27 negative. In Table III. are included all the results where the Flocculation reaction was weak but was associated with a positive Wassermann reaction and was classified as positive. Without exception, these cases were all syphilitic but mostly treated. In such cases weak reactions were to be expected. In cases 4, 12, 13 and 14, the flocculation effect was exceedingly weak and the results/

results are of interest in indicating how even the weakest reaction may be significant, taking into account also the results of the Wassermann reaction and clinical state. Table IV. shows the results in which the Wassermann reaction was definitely positive but the Flocculation reaction was quite negative, no flocculation occurring even in a 1 : 2 dilution. Table V. illustrates cases which exhibited a negative Wassermann test and a positive Flocculation test. Table VI. includes a series of tests in which the Wassermann reaction was doubtful but the Flocculation test was positive.

DISCUSSION

In any laboratory test for syphilis, the interpretation of the results is a matter of some importance as it is evident that great responsibility is incurred by the laboratory worker. It is obvious that there is little difficulty when both tests are in agreement - both frankly positive or negative - and this is illustrated in Table II. As regards the cases shown in Table III., the Flocculation reactions are weak but are supported by positive Wassermann reactions. Without exception, all were syphilitic cases but had been under treatment. Even such weak flocculation effects seemed, therefore, to be significant./

significant. In Table IV. marked reactions are shown in the Wassermann test while the Flocculation reactions are negative. Reference to the clinical state shows that the cases had been under treatment and 4 of the 5 were clinically cases of syphilis. Thirty-three cases were positive in the Flocculation test, and of these, 21 were negative and 12 were doubtful in the Wassermann reaction. Certain of the results were more distinct than those in Table III.; others were like those in Table III. Seventeen had been under treatment for syphilis, 3 were cases of untreated syphilis, 11 were mental cases and the re-:maining 2 were cases of gonorrhoea. It is of spec-:ial interest to note that 2 cases of gonorrhœa yielded positive results in both reactions while another case showed a positive result in the Wasser-:mann reaction only and 2 gave a positive result in the Flocculation test with negative and doubtful re-:sults respectively in the Wassermann reaction. As another case gave a positive Lange reaction (vide Section E.), the opinion has been formed that it is difficult to believe that cases of gonorrhoea which show such results have not a mixed infection, which is probably masked when the discharge is profuse, thus escaping clinical detection.

As regards the 4 cases, already referred to in Table III., taking into consideration the clinical state, one is forced to the conclusion that any change at/

at all in the Flocculation test, even the weak react-:ions shown in Tables III., V. and VI. may be signifi-:cant. If this observation is confirmed by other workers then it will greatly enhance the value of this simple test.

SUMMARY AND CONCLUSIONS

One thousand, two hundred and eleven sera from both syphilitic and non-syphilitic cases were carefully tested by the Wassermann and Flocculation methods. There was agreement in 1,173, i.e. 96·86 per cent., and disagreement in 38, i.e. 3·13 per cent. Of the latter, 0·41 per cent. yielded positive Wassermann reactions with negative Flocculation tests, while 2·76 per cent. gave positive Flocculation reactions with negative Wassermann tests. The discrepancies between the two reactions have been discussed and the general results tend to favour the Flocculation reaction as a reliable serum test in the diagnosis of syphilis. On the other hand, it must be recognised that where it is essential to obtain the maximum of serological inform-:ation in any particular case, both reactions should be applied, and even when concordant results are ob-:tained, the confirmation ensured in this way is of the utmost value. The Flocculation test, like many other laboratory tests, has, of course, its limitations. Attention has already been directed to the temperature factor in heating the serum preparatory to the react-:ion/

reaction and the influence of shaking, but particular attention should be given to the "age" of the serum. It is, therefore, of prime importance to carry out the test as soon after withdrawal of the blood as possible; after being kept for 4 days or longer, sera originally positive tend to yield zone reactions and may become negative. In certain treated cases of syphilis where the Wassermann reaction is negative, the Flocculation test gives weak positive results and it is suggested that in such cases this is an indic-:ation for continuation of treatment; as a matter of fact, in some of these cases both reactions became definitely positive when treatment was entirely sus-:pended. It would be advisable in such cases that both tests should be carried out with a view to ob-:taining the fullest information regarding the react-:ion of the serum. With these exceptions, substitut-:ion of the Flocculation test for the Wassermann re-:action could be recommended in the routine examinat-:ion of all recent untreated cases because the re-:sults of the former are quite as accurate as those of the Wassermann reaction.

The advantages of the Flocculation test (as carr-:ied out by the method described) have been discussed and depend on the simplicity of the technique as con-:trasted with the complement-fixation test. Though the routine serological examination of suspected cases of syphilis can be vastly simplified by the adoption of/

of this method, attention to detail in the technique is as essential as in any other serological method. In certain cases the combined application of both Flocculation and Complement-fixation tests is undoubt-:edly of the greatest value.

TABLE I.

Number of Cases	Positive in both reactions	Negative in both reactions	Wassermann reaction positive. Flocculation Test negative	Wassermann reaction negative. Flocculation Test positive	Wassermann reaction doubtful. Flocculation Test positive	Both tests in agreement	Non-agreement
1211	420	753	5 = 0·41 per cent.	21 = 1·76 per cent.	12 = 1·00 per cent.	1173 = 96·86 per cent.	38 = 3·13 per cent.

TABLE II.

	Wassermann reaction								Flocculation test							
	Test				Serum Con-:trol		Test with known negative serum		Serum dilutions							Number of hours after which readings made
Doses of Complement:	2	4	6	10	2	4	2	4	1 in 2	1 in 4	1 in 8	1 in 16	1 in 32	1 in 64	Con-trol.	
Number of Case																
1.	O	O	O	O	c.		c.		++++	++++	++++	++++	++++	++++	-	24
2.	O	O	O	O	c.		c.		++++	++++	++++	+++	+++	++	-	18
3.	O	O	O	t.	c.		c.		++++	++++	+++	+++	+++	++	-	12
4.	O	O	O	d.	c.		c.		++++	++++	++++	+++	++	+	-	14
5.	O	O	d.	j.c.	c.		c.		++++	+++	+++	++	+	+	-	24
6.	O	O	m.	c.	c.		c.		++++	++	++	+	+	-	-	24
7.	O	O	t.	j.c.	c.		c.		++++	+++	+	+	-	-	-	18
8.	O	O	d.	j.c.	c.		c.		++++	+++	+++	+	+	-	-	18
9.	O	t.	d.	c.	c.		c.		+++	++	+	-	-	-	-	24
10.	O	d.	a.c.	c.	c.		c.		+++	++	+	-	-	-	-	24
11.	O	m.	j.c.	c.	c.		c.		++	++	++	-	-	-	-	36
12./																

TABLE II. (Continued)

	Wassermann reaction								Flocculation test							
	Test				Serum Con-:trol		Test with known negative serum		Serum dilutions							Number of hours after which readings made
Doses of Complement:	2	4	6	10	2	4	2	4	1 in 2	1 in 4	1 in 8	1 in 16	1 in 32	1 in 64	Control	
Number of Case																
12.	O	m.	c.		c.		c.		++	+	+	-	-	-	-	18
13.	t.	v.m.	c.		c.		c.		++	+	+	-	-	-	-	36
14.	d.	m.	j.c.		c.		j.c.		++	+	+	-	-	-	-	36
15.	O	a.c.	c.		c.		c.		++	+	+	+	-	-	-	36
16.	a.c.	c.			c.		c.		-	-	-	-	-	-	-	36
17.	j.c.	c.			c.		c.		-	-	-	-	-	-	-	36
18.	c.				c.		c.		-	-	-	-	-	-	-	36
19.	m.	c.			c.		a.c.	c.	-	-	-	-	-	-	-	36
20.	v.m.	c.			v.m.	c.	a.c.	c.	-	-	-	-	-	-	-	36
21./																

TABLE II. (Continued)

	Wassermann reaction								Flocculation test							
	Test				Serum Con-:trol		Test with known negative serum		Serum dilutions							Number of hours after which readings made
Doses of Complement:	2	4	6	10	2	4	2	4	1 in 2	1 in 4	1 in 8	1 in 16	1 in 32	1 in 64	Control	
Number of Case																
21.	c.				c.		c.		-	-	-	-	-	-	-	36
22.	a.c.	c.			a.c.	c.	j.c.		-	-	-	-	-	-	-	36
23.	c.				c.		c.		-	-	-	-	-	-	-	36
24.	v.m.	c.			v.m.	c.	m.	c.	-	-	-	-	-	-	-	36
25.	m.	c.			v.m.	c.	a.c.	c.	-	-	-	-	-	-	-	36
26.	j.c.	c.			c.		c.		-	-	-	-	-	-	-	36
27.	c.				c.		c.		-	-	-	-	-	-	-	36

TABLE III.

Flocculation Test: weak positive
along with
Wassermann Reaction: positive

	Wassermann reaction								Flocculation test							
	Test				Serum Con-:trol		Test with known negative serum		Serum dilutions						Number of hours after which readings made	Clinical state
Doses of Complement	2	4	6	10	2	4	2	4	1 in 2	1 in 4	1 in 8	1 in 16	1 in 32	Con-trol		
Number of Case																
1.	d.	m.	c.		c.		c.		++	+	-	-	-	-	48	Syphilis (treated)
2.	d.	m.	j.c.		c.		c.		++	+	-	-	-	-	48	Congenital Syphilis
3.	t.	m.	c.		c.		c.		++	+	-	-	-	-	48	Syphilis (treated)
4.	d.	m.	c.		c.		c.		+	+	-	-	-	-	36	Syphilis (treated)
5.	d.	v.m.	j.c.		c.		c.		++	+	-	-	-	-	45	Latent Syphilis (treated)
6.	0	d.	j.c.		c.		c.		++	+	-	-	-	-	45	Congenital Syphilis (treated)
7.	0	m.	c.		c.		c.		++	+	-	-	-	-	35	Congenital Syphilis (treated)
8.	d.	a.c.	c.		d.	c.	c.		++	+	-	-	-	-	24	Tertiary Syphilis (treated)
9/																

TABLE III. (Continued)

Flocculation Test: weak positive
along with
Wassermann Reaction: positive

	Wassermann reaction								Flocculation test							
	Test				Serum Con-:trol		Test with known negative serum		Serum dilutions						Number of hours after which readings made	Clinical state
Doses of Complement	2	4	6	10	2	4	2	4	1 in 2	1 in 4	1 in 8	1 in 16	1 in 32	Con-:trol		
Number of case																
9.	ft.t.	v.m.	c.		c.		c.		++	+	-	-	-	-	36	Syphilis (treated)
10.	t.	v.m.	c.		c.		c.		++	+	-	-	-	-	36	Syphilis (treated)
11.	m.	v.m.	c.		c.		c.		++	+	+	-	-	-	36	Latent Syphilis (treated)
12.	0	t.	c.		c.		c.		+	+	-	-	-	-	46	Syphilis (treated)
13.	0	t.	v.m.	c.	c.		c.		+	-	-	-	-	-	24	Syphilis (treated)
14.	0	0	0	0	c.		c.		+	+	-	-	-	-	36	Syphilis (treated)
15.	0	a.c.	c.		c.		c.		++	++	-	-	-	-	24	Secondary Syphilis (treated)
16.	m.	a.c.	c.		c.		c.		++	+	-	-	-	-	45	Congenital Syphilis (treated)
17.	d.	v.m.	c.		c.		c.		++	+	-	-	-	-	48	Tertiary Syphilis (treated)
18.	t.	a.c.	c.		c.		c.		++	+	-	-	-	-	36	Syphilis (treated)

TABLE IV.

Wassermann Reaction: positive
Flocculation Test : negative

	Wassermann reaction								Flocculation test							
	Test				Serum Con-:trol		Test with known negative serum		Serum dilutions						Number of hours after which readings made	Clinical state
Doses of Complement:	2	4	6	10	2	4	2	4	1 in 2	1 in 4	1 in 8	1 in 16	1 in 32	Con-:trol		
Number of case																
1.	0	d.	a.c.	c.	c.		c.		-	-	-	-	-	-	48	Syphilis (treated)
2.	0	0	0	0	c.		c.		-	-	-	-	-	-	48	Syphilis (treated)
3.	0	0	0	0	c.		c.		-	-	-	-	-	-	36	Syphilis (treated)
4.	0	0	v.m.	c.	v.m.	c.	t.	j.c.	-	-	-	-	-	-	36	Gonorrhoea (treated)
5.	0	0	0	0	a.c.	c.	a.c.	c.	-	-	-	-	-	-	36	Syphilis (treated)

TABLE V.

Wassermann Reaction: negative
Flocculation Test : positive

	Wassermann reaction								Flocculation test							
	Test				Serum Con-:trol		Test with known negative serum		Serum dilutions						Number of hours after which readings made	Clinical state
Doses of Complement:	2	4	6	10	2	4	2	4	1 in 2	1 in 4	1 in 8	1 in 16	1 in 32	Con-trol		
Number of case																
1.	m.	c.			c.		v.m.	c.	++	++	++	+	-	-	36	Gonorrhoea (treated)
2.	c.				c.		c.		++	++	+	-	-	-	48	Syphilis (treated)
3.	c.				c.		c.		+++	++	+	-	-	-	12	Tabes (treated)
4.	j.c.				c.		j.c.		+	+	+	+	-	-	48	Syphilis (treated)
5.	c.				c.		c.		++	++	++	+	-	-	24	Syphilis
6.	a.c.	c.			c.		a.c.	c.	++	++	+	-	-	-	48	Latent Syphil-:is(treated)
7.	c.				c.		c.		++++	+++	++	+	-	-	48	Syphilis (treated)
8.	c.				c.		c.		++	++	+	+	-	-	48	Syphilis (treated)
9.	c.				c.		c.		+++	+	-	-	-	-	36	Tabes (treated)
10.	j.c.				c.		a.c.	c.	++++	++++	++++	+++	+	-	36	Feebleminded
11.	d.	c.			c.		m.	c.	++	++	+	-	-	-	36	Feebleminded
12./																

TABLE V. (Continued)

Wassermann Reaction : negative
Flocculation Test : positive

	Wassermann reaction								Flocculation test							
	Test				Serum Con-:trol		Test with known negative serum		Serum dilutions						Number of hours after which readings made.	Clinical state.
Doses of Complement:	2	4	6	10	2	4	2	4	1 in 2	1 in 4	1 in 8	1 in 16	1 in 32	Con-trol.		
Number of case.																
12.	a.c.	c.			c.		a.c.	c.	++	++	+	-	-	-	36	Manic-depress-:ive.
13.	v.m.	c.			c.		v.m.	c.	+++	+++	++	+	-	-	36	Melancholia
14.	m.	a.c.	c.		v.m.	c.	m.	a.c.	++	+	-	-	-	-	36	Imbecility
15.	m.	a.c.	c.		v.m.	c.	m.	a.c.	++	+	-	-	-	-	36	Dementia
16.	a.c.	c.			a.c.	c.	a.c.	c.	++	+	-	-	-	-	36	Stupor
17.	m.	c.			v.m.	c.	a.c.	c.	++	+	-	-	-	-	36	Paranoia
18.	m.	c.			m.	c.	m.	j.c.	++	+	+	-	-	-	36	Dementia
19.	m.	a.c.	c.		m.	c.	m.	a.c.	++	+	-	-	-	-	36	Dementia
20.	m.	c.			m.	c.	v.m.	c.	++	+	-	-	-	-	36	Dementia
21.	c.				c.		c.		++	+	-	-	-	-	36	Syphilis (treated)

TABLE VI.

Flocculation Test : positive
Wassermann Reaction: doubtful

	Wassermann reaction								Flocculation test							
	Test				Serum Con-:trol		Test with known negative serum		Serum dilutions						Number of hours after which readings made	Clinical state
Doses of Complement:	2	4	6	10	2	4	2	4	1 in 2	1 in 4	1 in 8	1 in 16	1 in 32	Con-trol		
Number of Case																
1.	m.	a.c.	c.		c.		a.c.	c.	+++	++	+	-	-	-	36	Tertiary syphilis (treated)
2.	d.	c.			c.		a.c.	c.	-	-	-	+++	+++	-	36	No active syphilis
3.	O	j.c.			c.		m.	j.c.	+++	++	-	-	-	-	24	Syphilis
4.	v.m.	c.			c.		c.		++	++	+	-	-	-	48	Syphilis (treated)
5.	m.	c.			c.		c.		+++	+++	+	-	-	-	48	Syphilis (treated)
6.	m.	a.c.	c.		c.		c.		+++	+++	+	-	-	-	36	Syphilis
7.	m.	c.			c.		j.c.		+++	+++	+	-	-	-	36	Syphilis (treated)
8.	d.	j.c.			c.		a.c.	c.	++	++	+	+	-	-	24	Syphilis (treated)
9.	d.	j.c.			c.		a.c.	c.	++	+	+	+	-	-	48	Syphilis (treated)
10.	m.	j.c.			c.		c.		++	+	-	-	-	-	48	Latent syphilis (treated)
11.	m.	a.c.	c.		c.		a.c.	c.	++	+	-	-	-	-	36	No active syphilis
12.	O	j.c.			c.		c.		++	+	-	-	-	-	36	Gonorrhœa (treated

SECTION D.

Other serological reactions applied in diagnosis of syphilis.

I N T R O D U C T I O N.

SECTION D.

Other serological reactions applied in diagnosis of syphilis.

I N T R O D U C T I O N.

In the previous section page 56, the writer re-:corded the results of a careful comparison of the Wassermann and Flocculation syphilis serum reactions. These favoured the Flocculation test and the practical application of this reaction was discussed. This work was later extended to a comparison of the Wasser-:mann and Flocculation tests with certain other serum reactions that have been advocated in syphilis diag-:nosis:- (1) the Colloidal benzoin reaction, (2) the Formol-gel test and (3) the Klausner reaction.

THE COLLOIDAL BENZOIN REACTION

Since the experiments of Zsigmondy (1901) which preceded the protein test devised by Lange (1912), various colloidal substances have been used for the detection of the syphilitic "reagin" in the serum and the cerebro-spinal fluid. Emanuel (1915) advocated the use of a colloidal solution of gum mastic, and Guillain, Laroche and Lechelle (1922) recommended the use of Colloidal benzoin. Luers (1920) and Ellinger (1921) have used certain metallic colloids but without much success. Cruickshank (1924) found the Colloidal benzoin test of less value than the Colloidal gold reaction; he considered that the only advantage of the/

the test, applied to the cerebro-spinal fluid, lay in its easy preparation and that it should only be used in the absence of a suitable gold sol preparation. Guillain, Laroche and Kudelski (1922) stated that the Colloidal benzoin test, applied to serum, did not yield results of any diagnostic value. Arnaud (1922) claimed that the Colloidal benzoin reaction could be applied to serum as well as cerebro-spinal fluid but that the reaction was slightly less delicate than the Flocculation test. Marie, Bouttier and Ivrgoulesco (1922) noted positive results in a diversity of cond-:itions, while Targowla (1922) concluded that the Colloidal benzoin reaction could not be utilized for serum-diagnosis and that both syphilitic and non-syphilitic sera gave positive reactions. Braxton-Hicks and Pearce (1924) considered the benzoin react-:ion nearly as sensitive as the Wassermann reaction, obtaining positive results in no diseases except syph-:ilis. Danila and Stroe (1923) stated that non-syphilitic and syphilitic sera reacted in approximate-:ly the same manner and to the same degree with Coll-:oidal benzoin. Mras (1922) claimed that the test was fairly sensitive. Recently Voge (1926) has re-:corded results in which the Colloidal benzoin test was in agreement with the Wassermann reaction in 89 per cent. and 82 per cent. of Wassermann positive and negative cases respectively.

Technique.- The technique of the test and the criteria/

criteria of a positive result described by Voge were followed. The benzoin sol was prepared according to the method described by Kermack and Voge (1925).

THE FORMOL-GEL REACTION.

Gaté and Papacostas (1920) described a test for syphilis which consisted in adding 2 drops of commercial formalin to 1 c.c. of clear unheated serum. The mixture was allowed to stand for 24 hours at room temperature. A positive result was denoted by the coagulation of the serum which did not occur in the case of non-syphilitic individuals. Ecker (1921) stated that the test was of no value because of its failure to react in syphilis and the occurrence of positive reactions with the sera of non-syphilitic individuals. Burke (1922) obtained negative results with sera which yielded positive results with the Wassermann reaction and all Wassermann positive cerebro-spinal fluids were negative. Suffern (1921) obtained similar results to Ecker. Ramakrishnan (1922) tested the sera of 539 cases sent for the diagnosis of syphilis along with the Wassermann reaction and the two tests were found to agree in only 66·6 per cent. of cases. Among sera from presumably active cases of syphilis the percentage of agreement was only 73·4. Armangué and Gonzales (1922) obtained positive results with sera from patients with malignant disease which gave negative Wassermann reactions and concluded that the/

the reaction was not due to some specific substance but to a relative increase in the usual constituents of normal serum. Holborow (1922) in a study of the conditions controlling gel formation concluded that the specificity of the formalin reaction was distinct-:ly open to question. Leger and Huchard (1922) had agreement with Wassermann results in only 47 per cent. while Bessemans (1922) concluded that the reaction was not specific for syphilis. Bessemans and Leynen (1922) obtained positive results with the sera of pigs, horses, cows, rats, rabbits, sheep, calves, guinea-pigs and dogs. Johnston (1923) stated that the re-:action failed to occur in definite cases of syphilis and noted positive reactions in non-luetic conditions. Gaté and Papacostas (1922) in a later comparison stat-:ed that the reaction was usually absent from normal sera but was not specific for syphilitic infection; they found it frequently positive in erysipelas and sometimes in gonorrhoeal infection. Kilduffe (1923) had negative reactions in certain cases of syphilis and positive in certain non-syphilitic persons. Reiner and Marton (1923) obtained more than half of their positive results from non-syphilitic sera.

Technique.— Clear, unheated serum and 40 per cent. formic-aldehyde were used for the tests. Into 4 agglutination tubes (3" × $\frac{1}{6}$") was placed 0·2 c.c. of patient's serum and varying amounts of formalin were added/

added to each tube - 0·01, 0·02, 0·03 and 0·05 c.c. The reagent and serum were mixed and the tubes allow-:ed to stand at room temperature for 24 - 36 hours when readings were taken. For the purpose of record-:ing results the occurrence of gel formation in any tube of the series has been classified as positive. As the reaction appeared to show no relationship to the Wassermann effect, any further study of its quant-:itative aspects was not pursued.

THE KLAUSNER REACTION

Klausner (1908) described a supposed syphilis reaction following the addition of 0·2 c.c. of clear unheated serum to 0·6 c.c. of distilled water; in about 15 hours a flocculent precipitate formed, marked in the case of syphilitic sera. While the reaction was originally regarded as due to an increase of serum globulins, Klausner (1912) at a later date suggested that an extraction of syphilitic serum with ether re-:moves the reacting substances and that the phenomenon was due to its containing lipoids. Hayn (1909) test-:ed 83 sera, all of which were negative in the Wasser-:mann reaction and 19 gave a positive Klausner react-:ion. Browning and Mackenzie (1924) have pointed out that febrile cases give a positive reaction. According to Citron (1908) and Sachs and Altmann (1907) heating at 55°C. or keeping the serum abolishes the reaction. A search through the literature of this

and/

and other countries failed to give much information on the subject and thus it was thought desirable to inquire further into the test.

Technique.- This is extremely simple. Freshly made distilled water was used throughout and the most convenient tube seemed to be the ordinary Wassermann tube (3" × ½"). In no test was serum used which was more than 3 days old after withdrawal. The tubes with 0·6 c.c. of distilled water and 0·2 c.c. of fresh serum - mixing thoroughly - were placed in the incub-:ator at 37°C. for 6 hours, allowed to stand at room temperature over-night and were then read. Precaut-:ions were taken to prevent bacterial contamination. A flocculent precipitate was regarded as a positive reaction.

WASSERMANN AND FLOCCULATION TESTS

The methods were those used in the Bacteriologi-:cal Department of the University of Edinburgh. These have already been described and discussed by the writer in previous papers (1925, 1926 and 1927).

RESULTS

One thousand three hundred and ninety-four sera have been used for comparative tests; in all cases the Wassermann and Flocculation reactions have been carried out, and among unselected groups of these sera, the/

the Colloidal benzoin, Formol-gel and Klausner reactions have been tested in parallel with the complement-fixat-:ion and Flocculation reactions. The general results are shown in Table I.

TABLE I.

Wassermann reaction (1,394)

Positive (310)							
F.T. (310)		C.B. (130)		F.G. (229)		K.R. (110)	
Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
305	5	44	86	143	86	61	49

Negative (1,084)							
F.T. (1084)		C.B. (506)		F.G. (884)		K.R. (348)	
Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
13	1071	5	501	54	830	21	327

F.T. = Flocculation test. F.G. = Formol-gel.
C.B. = Colloidal benzoin. K.R. = Klausner reaction.

Tables II. III. and IV. show the detailed compari-:sons with the Wassermann and Flocculation reactions of the Colloidal benzoin, Formol-gel and Klausner re-:actions respectively.

TABLE II.

	Positive			Negative.		
No. of cases examined	W.R.	F.T.	C.B.	W.R.	F.T.	C.B.
636	130	132 Pos. Neg. 128 2	49 Pos. Neg. 44 86	506	504 Pos. Neg. 4 502	587 Pos. Neg. 5 501

TABLE III/

TABLE III.

No. of cases examined	Positive. W.R.	F.T.	F.G.	Negative. W.R.	F.T.	F.G.
1,113	229	233 Pos. 227 Neg. 2	197 Pos. 143 Neg. 86	884	880 Pos. 6 Neg. 878	916 Pos. 54 Neg. 830

TABLE IV.

No. of cases examined	Positive. W.R.	F.T.	K.R.	Negative W.R.	F.T.	K.R.
458	110	114 Pos. 107 Neg. 3	82 Pos. 61 Neg. 49	348	344 Pos. 7 Neg. 341	376 Pos. 21 Neg. 327

D I S C U S S I O N

COLLOIDAL BENZOIN REACTION.

It will be seen from Table II. that while there is a very close correspondence between the Wassermann and Flocculation effects, a considerable proportion of Wassermann-positive sera failed to yield a positive Colloidal benzoin reaction, viz., 86 out of 130 (66·16 per cent.) Among the Wassermann negative sera a small percentage (5 out of 506) only were positive in the Benzoin test. The failure of this reaction lies in its lack of sensitiveness. Thus, though Wassermann negative/

negative sera generally react negatively in the benz-:oin test, only the most active Wassermann-positive sera gave a definite precipitation reaction with Coll-:oidal benzoin. The test fails, therefore, in the detection of sera which are weakly positive in the Wassermann reaction. The actual readings of the degree of precipitate present considerable difficulty and it has been found that the personal factor is a considerable element in this.

FORMOL-GEL AND KLAUSNER.

When gel formation occurred it was observed gener-:ally in all the tubes of the series; in some cases coagulation was observed in the first 3 tubes, i.e., no gel resulted from the addition of 0·05 c.c. forma-:lin; in one instance gel formation occurred only in tubes I. and II.

Tables III. and IV. show clearly the absence of any relationship between this effect and the Wassermann and Flocculation reactions.

CONCLUSIONS

1. The Wassermann and Flocculation reactions show exceedingly close agreement.

2. In the Colloidal benzoin reaction Wassermann-negative sera usually react negatively.

3. There is no correspondence between either the Formol-gel or Klausner reaction and the Wassermann reaction.

SECTION E.

The serological diagnosis of Neuro-Syphilis

INTRODUCTION

SECTION E.

THE SEROLOGICAL DIAGNOSIS OF NEURO-SYPHILIS.

I N T R O D U C T I O N

At the present time in the routine diagnosis of neuro-syphilis, various other laboratory tests, in addition to the Wassermann reaction, are being applied to the cerebro-spinal fluid; and the cytological examination of the fluid also constitutes an important diagnostic method. The correct diagnostic evaluation of these laboratory methods is, therefore, of obvious importance in the study of syphilitic disease of the central nervous system.

THE WASSERMANN REACTION with CEREBRO-SPINAL FLUID.

According to Dujardin (1921), the presence of the Wassermann reacting substance in the cerebro-spinal fluid of syphilitics is due to an increase of meningeal permeability and not to any intrameningeal formation of this antibody, and any form of aseptic inflammation of the meninges occurring in a syphilitic patient with a positive Wassermann may confer the reaction on the fluid. Weil and Kafka (1911) have shown that in inflammatory conditions hæmolysin and complement/

complement can pass through the blood vessels into the cerebro-spinal fluid. Complement has been found in the fluid in cases of general paralysis, in cerebro-spinal syphilis, in meningitis and in spinal tumour. Flexner and Amoss (1917) effected the passage of immune substances for the poliomyelitis virus from the blood into the cerebro-spinal fluid by injecting horse serum intrathecally. When the Wassermann reaction was first applied to the fluid few positive results were recorded. With the original Wassermann technique a positive result was rare. Thus Plaut (1909) obtained only 6 per cent. in his cases. With more delicate technique and the use of larger quanti-:ties of fluid the percentage of positive results has been greater. Plaut (1911) still claims that unless a relatively large amount of cerebro-spinal fluid is used in the test positive results may be missed even in cases of general paralysis. Generally with small amounts, e.g. 0·05 - 0·2 c.c., a much smaller percent-:age of positive results is obtained and negative results are frequently recorded if the test is carried out during active treatment. McIntosh and Fildes(1914), however, have stated that non-specific results are obtained/

obtained with quantities larger than 0·2 c.c. Other authors have found the cerebro-spinal fluid anticomplementary when used in large amounts. According to Browning and Mackenzie (1924), in carry-:ing out the Wassermann test it is unnecessary to heat the cerebro spinal fluid at 56° C. and they use the fluid instead of normal saline for preparing the antigen emulsion.

It is now well known that in some cases the cerebro-spinal fluid may react positively while the serum is negative. This may occur in (a) syphilis of the brain and (b) treated cases. According to Boas (1922), the cerebro-spinal fluid in the primary stage of syphilis is always negative. On the other hand, With (1917), Kyrle, Brandt and Mras (1920) have shown that in some cases of primary syphilis the spinal fluid is positive before the serum. Other authors have observed that syphilis attacks the pia-arachnoid at a very early stage and assert that changes can be seen in the cerebro-spinal fluid during the late primary and early secondary stages in 50 per cent. of all cases of syphilis. In secondary syphil-:is without cerebral symptoms the fluid, according to Plaut, Boas and Lind (1923) is always negative and only/

only cases showing cerebral involvement react positively. Collins and Craig (1914) have shown that the cerebro-spinal fluid is affected early and in a relatively large number of cases. Wile (1923) found that 30 per cent. of secondary cases gave a positive result. Kolmer (1923) considers that the Wassermann reaction with cerebro-spinal fluid has its greatest value in tertiary syphilis. In general paralysis the fluid is positive in 96 - 100 per cent. Mott (1916) records 5 negative results in a series of 300 cases of general paralysis. Plaut (1911) found 9 negative in his series of 276, while Boas (1922) noted 12 negative results in a series of 201 cases. Among other workers there has been greater variation in the results of the Wassermann reaction of the cerebro-spinal fluid in cases of general paralysis. Thus Marie and Levaditi (1907) obtained 73 per cent. positive; Marie, Levaditi and Yamanouchi (1908), 93 per cent.; Stertz (1923), 88·8 per cent; Noguchi and Moore (1910), 88 per cent; Smith and Candler (1923), 92·1 per cent; Marinesco (1919), 94 per cent; Noguchi, Rosanoff and Wisemann (1923), 87·5 per cent; Morgenroth and Stertz (1907), 100 per cent; and Nonne (1921) 100 per cent. Nonne states that if large amounts of fluid are used 100 per cent. positive results/

results can be obtained in locomotor ataxia and in cerebro-spinal lues. Boas and Neve (1916) found 2 negative cases in 19 of locomotor ataxia. Fildes and McIntosh (1913) in the examination of 60 cases of cerebro-spinal syphilis obtained positive results in 36 .

GLOBULIN TESTS

In a normal cerebro-spinal fluid there is but a trace of protein which consists mostly of globulin. In various pathological conditions there is an in-:crease but practically no attention has been given to the exact quantity of the proteins or their nature. Mestrezat (1912) holds that the serum albumin is mostly increased in acute inflammatory conditions, and the globulin is increased in syphilitic affections of the cerebro-spinal system.

Hektoen and Heymann (1920) have studied the action of specific antiglobulin and antialbumin precipitins on cerebro-spinal fluid and found that in general paralysis both the albumin and globulin are increased but principally the globulin. Though the increased protein represents more albumin than globu-:lin, most workers have given greater attention to the latter/

latter, and various globulin tests have been applied to the cerebro-spinal fluid, particularly in the diagnosis of neuro-syphilis. The recognised globu-:lin tests are those of Noguchi, Ross-Jones, Nonne-Apelt, Pandy, Kaplan and the sulphosalicylic-mercuric-chloride. The technique of these is referred to later. Most authors recognise that no test or group of tests is diagnostic of any disease of the central nervous system; indeed, Levinson (1923) lays much stress on the fact that all tests are at times un-:reliable unless supported by other evidence, and that no one test can be regarded as a final criterion. This author holds that the Noguchi, Ross-Jones and Pandy tests run parallel in the same case, and he found the sulphosalicylic-mercuric-chloride tests useful in the diagnosis of tuberculous meningitis; early in the course of the disease when other tests were negative these two gave positive results.

Grossmann (1925) examined 60 cases, including 28 of general paralysis, and he obtained a positive Wassermann reaction in all the paralytics, while the Nonne-Apelt, Weichbrodt (a modified Pandy test) and Boltz tests agreed with the Wassermann findings. In one case of locomotor ataxia all tests were negat-:ive. The remainder of his series consisted of cases/

cases of epilepsy, senile dementia, dementia praecox, manic depressive insanity and the psychoses due to arterio-sclerosis and alcoholism. In all of these cases the various tests were negative.

The Noguchi test has the disadvantage that the butyric acid reagent has a pungent odour and requires boiling. The Kaplan test has the same objection. The Ross-Jones and the Nonne-Apelt are tests in which globulin is salted out by ammonium sulphate.

The Ross-Jones test seems to be more used in this country than any of the others because it has no odour and does not require boiling. The Pandy, sulphosalicylic-mercuric-chloride tests have been found by most workers to give reliable results.

LANGE REACTION

Since Lange (1912) introduced the colloidal gold test, much has been written appraising its value in the diagnosis of syphilis of the central nervous system.

Zsigmondy (1901) found that a solution of protein precipitated colloidal gold in the absence of an electrolyte. In his earlier experiments, Lange found in a case of general paralysis that the cerebro-spinal/

cerebro-spinal fluid (which contains much albumin) instead of showing a protective effect, as Zsigmondy had observed in the case of other albuminous sub-:stances, precipitated the goldsol in the form of metallic gold. Continuing his experiments, he showed that in tabes and in acute meningitis the cerebro-spinal fluid also precipitated the colloidal gold solution and he differentiated types of reaction designated "paretic", "tabetic" and "meningitic" by means of a series of dilutions of the fluid varying according to the strength of the reaction when tested quantitatively, i.e. with a series of varying dilut-:ions (vide infra methods). Lange considered that by his method it was possible to measure the protein content of spinal fluid according to the degree of precipitation.

The German school has long relied on the four reactions of Nonne (1910) in the diagnosis of syphilis of the nervous system :-

(1) A positive Wassermann reaction of the serum.

(2) A positive Wassermann reaction of the cerebro-spinal fluid.

(3) Globulin increase.

(4) Pleocytosis, to which the Lange test was added.

Miller, Brush, Hammers and Felton (1915) confirmed/

confirmed Lange's work and showed that the "paretic" type was constant in general paralysis but that the "tabetic" type, now termed the "luetic" curve, was common to other syphilitic conditions; and also found that such disease as disseminated sclerosis also gave paretic and luetic curves. Finally they concluded that not only the paretic curve but also the luetic curve were found in non-syphilitic condit-:ions. Other workers have found the paretic curve in cases of disseminated sclerosis, in encephalitis lethargica, in syphilitic meningo-myelitis and in tabetics with no mental symptoms.

Felton (1917), Fischer (1921), Weston (1920) and, more recently, Anwyl-Davis and Mellanby (1923) with other workers, agree that the globulin is re-:sponsible for precipitating the colloidal gold. They conclude that the various types of reaction depend on the relative amounts of albumin and of globulin.

German authors conclude that in cases which present the syndrome of Froin (1903) the albumin masks the reaction except in the higher dilutions and there the change is more one of colour than anything else and never more than purple or blue - this they term "Rechtsverschiebung".

Levinson/

Levinson (1919) found increase of albumin in cases of epilepsy which may account for the number of cases which were undoubtedly due to congenital syphil-:is.

Warwick and Nixon (1920) in an examination of 800 cerebro-spinal fluids from patients suffering from mental and nervous affections conclude that while the various curves are not diagnostic they are of significance in cerebro-spinal syphilis if taken in conjunction with other laboratory tests. These authors obtained 95 per cent. positive results in the colloidal gold test and 90 per cent. positive Wasser-:mann reactions with the fluid of general paralytics. In tabes the fluid gave 80 per cent. positive with the goldsol test and 55 per cent. with the Wassermann reaction. In cerebro-spinal syphilis they obtained 75 per cent. while in 18 per cent. of their cases the Lange reaction was the only positive indication of abnormality in the fluid.

Eicke (1913) examined 300 cases and obtained typical reactions in dementia paralytica, secondary syphilis and meningitis.

Lee and Hinton (1914) obtained colloidal gold reactions in 24 cases of tabes, 9 of which gave nega-:tive Wassermann reactions with serum and cerebro-spinal fluid and of these, two gave no other positive test/

test with the fluid. In 8 cases without involvement of the nervous system, they obtained 4 positive colloidal gold reactions, 4 positive Wassermann reactions with the serum, but negative results with the cerebro-spinal fluid.

Miller and Levy (1914) examined 210 fluids and found that all cases of general paralysis and tabes gave paretic and luetic reactions respectively; luetic reactions were obtained in 13 out of 15 cases of latent syphilis in which there was no clinical evidence of involvement of the central nervous system, and in 80 per cent. of congenital syphilis. They were convinced that the test was more valuable in differentiating the various syphilitic lesions of the nervous system but were of opinion that it had no advantages over other laboratory tests in congenital, secondary and tertiary syphilis and that it did not distinguish between tuberculous and suppurative meningitis.

Grulee and Moody (1913) disagreed with the findings of Miller and Levy in regard to the value of the gold test in congenital syphilis and drew attention to the fact that these had obtained a positive Wassermann reaction in 100 per cent. in this condition./

condition.

Lowrey (1917) obtained no positive reactions in non-syphilitic mental cases, but reported 10 per cent. of atypical results.

McCowan (1925) in a recent paper examined 60 fluids from mental cases. Thirty-seven of the 40 cases of general paralysis gave the paretic type of reaction, while the other 3 tended to it.

THE FOAM TEST

This test does not appear to have been applied to any extent in this country. It depends on the formation and persistence of a foam layer on shaking the tubes; this is of course due to increase of protein. The test has been much used by Levinson (1922) and his co-workers. He claims that it is positive in all pathological fluids but is more marked in acute infections of the meninges. Zingher (1917) describ-:ed the formation of a heavy foam in acute poliomyelit-:is, thus corroborating the earlier findings of Levin-:son.

CYTOLOGY

Ravaut, Sicard and Widal (1903) were the first to show the diagnostic significance of the cell con-:tent and since then this method of investigation has been/

been strongly advocated by others.

Most observers agree with the statement that normal cerebro-spinal fluid seldom contains more cells than 3 per c.mm. Fuchs and Rosenthal (1923) found 0 - 2 cells per c.mm. Rehm (1923) found 1 - 5 and even 6 - 9 per c.mm. Gennerich (1923) found 8 in the fluid of one healthy person; others give the numbers 5 - 20. Levinson (1923) regards the normal fluid as containing 1 - 6 cells per c.mm. and a count over 6 as suspicious and over 10 as indicative of some pathological condition. He does not regard every increase of cells as evidence of inflammation of the meninges. Fischer (1921) claims that the cell count obtained by lumbar puncture does not represent that of the cerebro-spinal fluid con-:tained in the ventricle of the brain. On the other hand, Nonne (1924) found no difference.

Normally no blood cells are present and the type of leucocyte is the small lymphocyte. Large lymphocytes occur infrequently but their presence raises suspicion. Polymorphonuclear cells are never present normally unless as a result of blood admixture in drawing the fluid.

In syphilitic conditions the increase of cells is due to small lymphocytes. Nonne (1921) observed lymphocytosis in 95 per cent. of general paralysis, in/

in 90 per cent. of tabes and in 100 per cent. of cerebro-spinal lues.

In pre-Wassermann days Ford Robertson believed that the laboratory diagnosis of general paralysis depended on lymphocytosis and the presence of plasma cells.

Ravaut (1907) found pleocytosis in 70 per cent. of cases of secondary syphilis - cases which exhibit-:ed no evidence of nervous involvement. Plaut (1911) also noted an increase of cells in the fluid in early syphilitic infection. Altmann and Dreyfus (1913) in 8 primary and 50 secondary cases found pleocytosis in 66 per cent. Zaloziecki and Fruhwald (1912) in an examination of 30 cases of early syphilis recorded 4 with 11-24 cells per c.mm., 5 with 22-87 and 2 with 94-212. Bergl and Klausner (1912) with 26 cases, of which 4 were in the primary stage, found 17 with pleo-:cytosis (two of which were primary).

Herrick and Dannenberg (1919) have observed pleocytosis and increase of globulin in cases of pneumonia, influenza, tonsillitis and the exanthems - all of which showed no evidence of meningitis.

Kyrle (1920) noted pathological changes in the fluids of 191 prostitutes, of which 126 gave a positive Wassermann reaction. One hundred and seventeen were examined by neurologists and only 50 showed evidence of/

of nervous involvement. He concludes from his examination of pathological fluids that all cases do not suffer later from disease of the central nervous system.

METHODS USED IN THIS INVESTIGATION.

In considering the value of any laboratory tests, correlation with clinical data is necessary. In certain well defined conditions the clinical evidence is quite sufficient for diagnostic purposes; in others the personal history is suggestive and sometimes the laboratory tests alone reveal the nature of the disease, but frequently it is necessary to correlate the patient's history, clinical data and the results of laboratory investigation. Even then cases are met with which leave much doubt as to the diagnosis. While certain laboratory tests are known to be more conveniently carried out and more reliable than others, some being simple, some complicated, there is always a danger in placing too much reliance on the more complicated reactions and for this reason the writer decided to make a critical investigation of tests used for serological diagnosis of syphilis as applied to the cerebro-spinal fluid. A series of 350 cases were investigated as regards personal history, clinical condition*, and the various laboratory tests referred/

*In the purely clinical investigations the writer was associated in all cases with another clinician.

referred to. Team work has been avoided in order to limit as far as possible the observations to one worker. The laboratory tests were :-

(1) Wassermann reaction of
    (a) the patient's serum.
    (b) the cerebro-spinal fluid.

(2) The colloidal gold test with the cerebro-spinal fluid.

(3) The Foam Test.

(4) Various tests for increase of globulin.

(5) Enumeration of cells, including in most cases a differential count.

The globulin tests performed were the :-

(a) Noguchi.
(b) Ross and Jones.
(c) Nonne and Apelt.
(d) Pandy.
(e) Kaplan.
(f) Sulphosalicylic acid.
(g) Mercuric-chloride.

The imvestigation is naturally divided into two parts, of which the first part deals with syphilit-:ic and the second part with non-syphilitic cases. Except in two cases the fluid was withdrawn by the author and the following is a brief description of the technique employed in cisternal and ventricular puncture :

CISTERNAL PUNCTURE

To Sir James Purves-Stewart I am indebted for

a/

a demonstration of the technique of obtaining fluid from the cisterna magna and this description has particular reference to his method. He believes the fluid obtained represents a more faithful index of the constituents than fluid obtained by lumbar punct-:ure because the cisterna magna contains more fluid than the terminal end of the sac. He considers also that this operation is quite safe when carried out by a skilled operator. Other exponents of this method are Wedgeforth, Ayer and Essick (1919).

Technique : A spinal puncture needle which has been sterilized is used and the stylet is kept in position. The thumb of the left hand is placed on the spine of the axis; there is a depression above this just below the occipital protuberance. The needle is inserted in the mid line just above the thumb and pushed cautiously forwards and upwards in a plane passing through the upper border of the ex-:ternal auditory meatus and the glabella. For be-:ginners it is as well to have graduated markings on the needle ranging from 3 to 6·5 c.m. In the aver-:age adult the cisterna is reached at a distance of 4·5 c.m. - a little less for a thin subject and a little more for a stout person. When the needle reaches the cisterna one soon learns to appreciate the feeling of negative resistance, and with some practice/

practice beforehand in the mortuary one need not hesitate to adopt this method. The stylet is then removed - 5 drops are allowed to escape lest there be any blood contamination - and as a further pre-:caution the fluid is collected into 3 sterile tubes already labelled I., II. and III.

VENTRICULAR PUNCTURE

This technique is not recommended for routine purposes except in the case of very young children who are not likely to live long or at least are the sub-:ject of gross mental defect. In all cases the anterior fontanelle was open.

Technique : The part should be shaved and sterilized and the needle treated as before with sty-:let in position. The child is laid flat on a table without a pillow and the head close to the edge. It is not necessary to give either a general or a local anaesthetic but the head should not be allowed to move during the operation. Lewkowicz (1924) does not recommend a local anaesthetic, and in the type of case described an anaesthetic is neither necessary nor desirable. It seems hardly necessary to mention that one must avoid the superior longitudinal sinus. The/

The needle is passed in a downward and slightly outward direction from the laternal angle of the fontanelle and the ventricle in an infant is reached at 4 c.m. from the surface. In marked hydrocephalus the distance is much less. Three specimens are taken into labelled tubes. It should be noted that there is generally a rise in temperature after the operation which subsides in 24 hours.

Every precaution was taken to see that no tests were carried out with fluids which contained blood and in order to obviate this, as already mentioned, the first five drops were allowed to escape, and in addition the fluid was run into three separate sterile tubes. All tests were carried out immediately and before autolytic changes set in.

WASSERMANN REACTION

The Wassermann reaction was carried out by a modification of the original method. The antigen was an alcoholic extract of heart muscle from a case of congenital syphilis. The extract was saturated with cholesterin and for the test a 1:10 emulsion was prepared by slow admixture so as to form a markedly turbid suspension. Fixed amounts of antigen (0·5 c.c.) and patient's serum (0·05 c.c.) heated at 55° C. for

half/

half an hour were tested with varying doses of guinea pig complement and 0·5 c.c. of hæmolytic system (sensitized ox red cells) was used. Known negative and positive control sera were always in-:cluded in each set of tests. The other necessary controls (antigen and serum) were also inclused. Sera and cerebro-spinal fluids for purposes of uniformity were inactivated for half an hour. The cerebro-spinal fluid was substituted for normal saline in making the antigen emulsion.

COLLOIDAL GOLD REACTION

With colloidal gold as well as with other laboratory reagents several workers have attempted standardisation. Certain workers have laid much stress on the importance of using doubly and trebly distilled water, the quality and the cleansing of the glass ware, the avoidance of rubber stoppers, etc. The writer believes that too much attention has been devoted to such unimportant details and too little given to the atmospheric conditions of the room in which reactions are carried out. The colloidal gold solution is extremely sensitive to acids in the atmosphere (as obtain in large cities), to acid pro-:ducts of combustion in the laboratory and to carbon-dioxide from other sources. As a preliminary a large number/

number of tests were carried out with Lange's fluid, with Levinson's modification and with a preparation obtained by the method of Miller, Brush, Hammers and Felton (1915), and it was found that all fluids were reliable in paretic patients but gave varying results with weak positive syphilitic and non-syphilitic meningitic cases. Black, Rosenberg and McBride (1917) had shown that oxalic acid used to neutralise the potassium carbonate in the method of Miller and his co-workers, interfered with the reducing action of the formalin and produced acid colloidal gold solutions. These critics give minute details of methods for testing the fluids and in adjusting acid and alkaline solutions, but it was found that even successful fluids made by the methods they so strong-:ly advocate, failed to give the reaction. It has been found that if, instead of using a two per cent. potassium carbonate and a one per cent. oxalic acid, a one per cent. neutral potassium oxalate is sub-:stituted then optimum results are obtained, and chances of producing an unsuitable colloidal gold are reduced to a minimum.

Technique : Into the first tube in a series of 10 is placed 0·9 c.c. of a 0·4 per cent. saline solution while to the remainder 0·5 c.c. is added.

To/

To tube I. - 0·1 c.c. of cerebro-spinal fluid is added, mixed and 0·5 c.c. of the mixture is transferred to tube II. This process of dilution is carried out till the end of the series is reached and the surplus 0·5 c.c. is rejected. Thus the dilutions range from 1:10 to 1:5120; an eleventh tube is added as a con-:trol and into it 0·5 c.c. of 0·4 per cent. saline is placed. To all 2·5 c.c. of colloidal gold is added and readings are taken in 18 hours. In positive reactions various changes take place -

(a) Complete flocculation - Supernatent fluid is water clear.
(b) Almost complete - Pale or greyish-blue colour.
(c) Marked - Blue colour.
(d) Distinct - Lilac or purple colour
(e) Trace - Reddish-blue colour.

In negative cases there is no change in any tube.

In the paretic type of reaction the gold is completely precipitated in the first 5 - 6 tubes and lesser changes in some or in all of the others, this being known as the "paretic curve" of Miller and Levy (1914). In the luetic type the flocculation is hardly ever complete and is greatest in the tubes 3 - 5, being much less or absent in the first two and in the higher dilutions: whereas the meningitic type shows the greatest/

greatest changes in tubes 6 - 8, the so-called "Verschiebung nach oben".

THE FOAM TEST

This test, like that of the colloidal gold reaction, is a test for excess of protein but depends more on the increase of albumin than of globulin. In all pathological fluids if the fluid is vigorously agitated for two minutes a thick layer of foam appears and persists for 20 minutes. In non-pathological conditions a very thin layer of foam develops on shaking and disappears within 5 minutes.

GLOBULIN TESTS

The Noguchi Test. The original test was used as it was found more reliable than the newer modified test.

Reagents : 1. Five cubic centimetres butyric acid were added to 45 c.c. of 0·85 per cent. salt solution.

2. Four per cent. solution of sodium hydrate.

Test : 0·2 c.c.of cerebro-spinal fluid is placed in a small test tube; 0·5 c.c. of reagent I. is added, boiled and 0·1 c.c. of reagent II. added and again boiled. A positive result is evidenced by flocculation. No result was regarded as positive where/

where there was no deposit within half an hour.

The Ross-Jones Test. This test is more popular in this country than any other but it may be remarked that few workers adopt the correct procedure in preparing the solution. If the solution is prepared by boiling as suggested by Levinson (1923) or by Browning and Mackenzie (1924) SO_2 is driven off, leaving free H_2SO_4. The water should not be heated above 60° C.

Reagent : Eighty-five grams of ammonium sulphate are dissolved in 100 c.c. water at 60° C. thus giving a saturated solution which should be filtered before use.

Test : This is carried out by superimposing an equal amount of the reagent on the cerebro-spinal fluid and in positive cases a white ring forms at the junction of fluid and reagent.

The Nonne-Apelt Test. This is a modification of the Ross-Jones test with the same reagent and in the same proportion as in the former test but the reagent and cerebro-spinal fluid are mixed together. A precipitate of globulin denotes a positive result.

The Pandy Test. This test, like the two preceding, does not require heating and there is no objectionable odour.

Reagent : One part of pure phenol is dissolved in 16 parts of distilled water.

Test : 0·1 c.c. of cerebro-spinal fluid is added/

added to 1 c.c. of the reagent in a test tube. In positive results a cloudy precipitate forms.

The Kaplan Test. This reagent, like the Noguchi, has a disagreeable odour which penetrates to every part of the room. It should be kept in a fume chamber.

Reagents : 1. Five per cent. butyric acid in 0·85 per cent. salt solution.

2. A saturated solution of ammonium sulphate.

Test : In a test tube 0·5 c.c. of cerebro-spinal fluid is boiled and 0·2 c.c. of reagent I. and 0·4 c.c. of reagent II. are added. A reading is made in 15 minutes. In positive cases there is a precipi-:tate.

The Sulphosalicylic Acid Test. This re-:action and the next are generally read together and the tubes are kept for 24 hours to compare the amount of precipitate.

Reagent : A three per cent. solution of sulpho-salicylic acid.

Test : 1 c.c. of cerebro-spinal fluid is placed in a test tube and 1 c.c. of the reagent is added. In positive cases there is a precipitate.

The Mercuric-chloride Test. After this test has been carried out the tube is set aside for 24 hours/

hours and comparative readings are made between this and the previous reaction.

Reagent : A One per cent. solution of mercuric-chloride.

Test : 1 cc. of cerebro-spinal fluid is placed in a test tube and 1 c.c. of reagent is added. If positive there is a precipitate.

According to Levinson (1923) in cases of suppurative meningitis the precipitate with sulpho-salicylic acid at the end of 24 hours may be double that of the mercuric-chloride test, while the opposite occurs in tubercular meningitis and in general para-:lysis.

The Weichbrodt Test. Mercuric-chloride is used as a 1 in 1000 solution. Three parts of this solution are added to seven of the cerebro-spinal fluid and in positive cases cloudiness results. After considerable experience with this test it was discarded in favour of that with the stronger solut-:ion and the results of the test are not incorporated.

CELL CONTENT

In the cerebro-spinal fluid most cells are easily recognised. In pathological fluids one may observe -

(1) Lymphocytes.
(2) Large mononuclears or hyaline.
(3)/

(3) Polymorphonuclears.
(4) Eosinophils.
(5) Plasma cells.
(6) Compound granular cells.
(7) Fibroblasts.
(8) Macrophage cells.*

Plasma cells may be confused with large mononuclears. To the late Dr. Ford Robertson, Pathologist to the Scottish Asylums, I owe a debt of gratitude for the time he spent and for the pains he took to make me familiar with this type of cell while I was a worker in his laboratory. The cell is not easily distinguished from the large hyaline cell: nucleus is rounded, eccentric, showing triangular condensations of chromatin spaced at intervals round its border; cytoplasm takes on a faint tinge with basic dyes. Pappenheim's stain (1907) is selective for plasma cells and shows up the beautiful clock-face arrangement of the chromatin in the nucleus. This cell is never found in a normal fluid. In all cases the cell enumeration was carried out before any changes had set in but in the case of trypanosomiasis investigated (vide infra) 6 drops of dilute acetic acid had previously been added to preserve the cerebro-spinal fluid in transit. In order to have uniform results the Fuchs-Rosenthal chamber was used which is 16 mm./

*The macrophage cell is a large mononuclear cell which in infective conditions was found to contain bacteria.

16 mm. square and 0·2 deep. The cells in all 16 squares were counted and the result gives the number of cells in 3·2 c.mm. of diluted cerebro-spinal fluid.

Technique :- A staining fluid was prepared which consisted of methyl violet 0·2 gm., Glacial acetic acid 5 c.c. and water to 100 c.c. The white cell pipette was filled with staining fluid to mark I. and cerebro-spinal fluid to mark XI. As the mixture contained 10 of cerebro-spinal fluid to 11 of the volume the result was multiplied by $^{11}/_{10}$. Three successive counts were made and the average taken. From a study of the literature it is not clear that all workers adopted the same methods of enumerating the cells, which may be accepted as the explanation for such divergent results. Thus the French method of counting or the use of the Thoma-Zeiss haemocyto-:meter gives different results from the more accurate Fuchs-Rosenthal method so much so that the cytological results of various workers are of little value for comparative purposes except that each result is serviceable in so far as it shows a pleocytosis.

RESULTS

I. Syphilitic cases - Of the 350 cases examined (detailed in Table I.), all the laboratory tests were done in a series of 329, while on the remainder the tests unfortunately had to be restricted owing to small/

small amounts of fluid available and to the fact that some of the specimens were not sufficiently fresh for cell counts. One hundred of these 329 cases were clinically syphilitic, and the Wassermann reaction was positive with sera in 96 and with cerebro-spinal fluid in 97. Few had been under treatment, with the exception of 5 cases of chronic syphilitic meningitis, three of which gave negative Wassermann reactions with serum and cerebro-spinal fluid, but it is of interest to note that these 3 cases each gave positive reactions in all tests one month after all treatment had been suspended. These 3 cases are, therefore, included in the 100 cases of syphilis. The following table gives an analysis of the results:-

TABLE

		Positive	Negative	Doubtful
Wassermann reaction	with cerebro-spinal fluid	97	3	0
	" serum	96	3	1
Colloidal Gold reaction	" cerebro-spinal fluid	89	11	0
Globulin tests	" " " "			
	Sulphosalicylic acid	95	5	0
	Mercuric-chloride	95	5	0
	Pandy (phenol)	94	6	0
	Noguchi (butyric acid)	86	14	0
	Ross-Jones (ammonium sulphate)	71	29	0
	Nonne-Apelt(" ")	71	29	0
	Kaplan (butyric acid + ammonium sulphate)	71	29	0
Foam test	.. ..	100	0	0

Cytology/

Cytology - Cells ranged from 9 to 442 per c.mm., and the table illustrates how cells varied (Page).

The Wassermann reaction gave negative results in the 3 cases of syphilitic meningitis, already re-:ferred to, and a doubtful result was obtained with the serum in one case of neuro-syphilis - a case which presented some indefinite symptoms pointing to early general paralysis but not definite enough to include it in that category.

The colloidal gold reaction was negative in 2 cases of early general paralysis, in one case of interstitial keratitis, in one of tabes dorsalis, in one of congenital syphilis, in one of latent, in 3 cases of syphilitic meningitis, in one of neuro-syphilis and one of secondary syphilis.

The sulphosalicylic acid and mercuric-chloride tests gave negative results in 5 instances; 2 cases of hydrocéphalus, one of congenital syphilis and 2 of syphilitic meningitis.

The Pandy test was negative in one case of secondary syphilis, 2 of hydrocephalus, one of con-:genital syphilis and 2 of syphilitic meningitis - 6 cases.

The Noguchi test failed in the following 14 :- 2 cases of congenital syphilis, 6 secondary syphilis, 1 neuro-syphilis, 3 hydrocephalus and 2 syphilitic meningitis.

The/

The Ross-Jones gave negative results in 5 cases of congenital syphilis, one of mental defic-:iency, 8 of secondary syphilis, one of neuro-syphilis one of interstitial keratitis, 4 of hydrocephalus, 3 of latent syphilis, 3 of tabes dorsalis and 3 of chronic meningitis - 29 cases.

The Nonne-Apelt and Kaplan showed each 29 failures also. In the former these were made up of 4 cases of congenital syphilis, 9 secondary, 2 neuro-, one interstitial keratitis, 4 hydrocephalus, 2 tabes dorsalis, one mental deficiency, one pyloric stenosis, one atrophic rhinitis and 4 syphilitic meningitis.

In the Kaplan the negatives included 3 cases of congenital syphilis, 9 secondary, one neuro-, one interstitial keratitis, 3 of hydrocephalus, 4 tabes dorsalis, one mental deficiency, one pyloric stenosis, 2 latent syphilis and 4 syphilitic meningitis.

An incomplete series of tests were carried out with 21 cases, the Wassermann reaction in all, the colloidal gold test in 19, one or more globulin tests and cell enumeration in 12. Most of these cases had been under treatment. The results are recorded in the following table :-

Table/

	Positive	Negative	No.of Cases
Wassermann Reaction with serum	13	6	21
" cerebro-spinal fluid.	13	8	21
Colloidal Gold Test " " "	9	10	19
Globulin Test " " "	11	1	12
Cells	11	1	12

Of these 21 cases, 2 were non-syphilitic (gonorrhœa), 4 were cases of primary syphilis,(3 of which had every test negative and were apparently cured, while the other gave negative Wassermann re-:actions with serum and cerebro-spinal fluid and a negative colloidal gold test; cells were not enumer-:ated nor were globulin tests done but it is of interest to note that, though the Sachs-Georgi flocculation test has formed part of another extensive investigation and is not yet completed, positive results were obtained with both serum and cerebro-spinal fluid). With 13 cases positive and other 6 accounted for, 2 more fall to be dealt with. The Wassermann reaction with the serum gave a negative result in a case of general paralysis and a case of latent syphilis, while with the fluid 2 latent cases were negative.

The colloidal gold test was not done in 2 cases/

cases of neuro-syphilis which gave positive Wassermann reactions with serum and cerebro-spinal fluid. A positive result was recorded (luetic type) in a case of gonorrhoea where every other test was negative. The second case of gonorrhoea gave a negative result as also did 4 cases of primary syphilis, 4 cases of latent syphilis and a case of secondary anaemia with slight jaundice.

Globulin tests were done in 12 instances but not the full series. A negative result was recorded in a case of treated primary syphilis which was nega-:tive with every other test. In this case cells were 1 per c.mm. Pleocytosis was present in the remaining 11 cases examined, cells ranging from 10 - 58.

As a result of this analysis 15 cases of syphilis fall to be added to the former 100, giving a total of 115. The Wassermann reaction was done in all but the colloidal gold test was done in 2 less and the following table summarises the results:-

		Positive	Negative	Doubtful
Wassermann Reaction	with cerebro-spinal fluid	110	5	0
	" Serum	109	5	1
Lange Reaction	" Cerebro-spinal fluid	97	16	0

The figures in the other tests remain unaltered but/

but the table shows the comparison between Wassermann and Lange tests.

Wassermann Reaction	with cerebro-spinal fluid is positive in	95·65	per cent.
"	with serum		
"	is positive in	94·78	" "
Lange Reaction	with cerebro-spinal fluid is positive in	85·84	" "

The following table gives the cell counts in the syphilitic cases :-

Cases		Average number of Cells per c.mm.	Cells varied between
1	Atrophic rhinitis	20·00	20 -
1	Banti's disease	104·00	104 -
1	Cerebral gumma	32·00	32 -
15	Congenital heart disease	46·10	19 - 100
2	" Pyloric stenosis	16·50	14 - 19
1	Eczema oris	19·00	19 -
1	Epilepsy	29·00	29 -
23	General paralysis	121·69	23 - 270
10	" " (early)	230·01	22 - 442
2	" " (Juvenile)	30·50	19 - 42
3	Hydrocephalus	39·00	19 - 68
1	Icterus neonatorum	21·00	21 -
1	Idiot	139·00	139 -
2	Imbecile	26·00	19 - 33
2	Interstitial keratitis	41·00	15 - 67
2	Secondary anaemia	33·00	33 -
5	Syphilitic meningitis	14·20	10 - 18
2	Syphilis Condylomata	11·50	9 - 14
11	" Congenital	22·18	12 - 39
7	" Latent	18·33	10 - 39
6	" Neuro-	57·75	13 - 100
4	" Primary	1·00	0 - 1
9	" Secondary	20·44	9 - 64
7	Tabes dorsalis	15·57	12 - 22
119			

It is specially noteworthy that as a result

of/

of this investigation with syphilitic cases the foam test stands first in respect of the constancy of positive results (100 per cent.). The Wassermann reaction, The Pandy and Sulphosalicylic-mercuric-chloride tests all correspond fairly closely, with a very high percentage of positive results. The Noguchi and Lange tests may be classified together, and the Ross-Jones, Nonne-Apelt and Kaplan form a class in which the percentage of positive results is lowest. The tests might thus be classified as follows :-

		Percentage of Positive Results.
Class I.	Wassermann reaction, Pandy, Sulphosalicylic-mercuric-chloride tests	94 - 100
" II.	Noguchi and Lange tests .	about 86
" III.	Ross-Jones, Nonne-Apelt and Kaplan tests	about 71

The detailed list of syphilitic cases (119) is shown in Table II. A synopsis of these cases follows; in it is included a case of trypanosomiasis, making the total number now dealt with 120. Negative cases are considered when they occur in the same group.

ATROPHIC RHINITIS.

Cases/

Cases - One.

History - Negative.

Clinical examination - Positive.

Lange reaction - Negative.

Wassermann reaction - Positive with serum and cerebro-spinal fluid.

Globulin tests - Noguchi, Pandy, Kaplan and Sulphosalicylic-mercuric-chloride positive; Ross-Jones and Nonne-Apelt negative.

Foam test - Positive.

Cells - 20 per c.mm. Practically all lymphocytes.

BANTI'S DISEASE.

Cases - One.

History - Negative.

Clinical examination - Evidence of congenital syphilis.

Lange reaction - Negative.

Wassermann reaction - Positive with serum and cerebro-spinal fluid.

Globulin tests - All positive.

Foam Test - Positive.

Cells - 104 per c.mm. - Lymphocytes - 95 per cent.
Large mononuclears - 4·5 " "
Plasma cells - 0·5 " "

CEREBRAL GUMMA

Cases - One.

History of syphilis while a soldier in India 25 years ago.

Clinical examination - Not conclusive.

Lange reaction - Negative.

Wassermann/

Wassermann reaction - Positive with serum and cerebro-spinal fluid.

Globulin tests - All positive except Ross-Jones and Nonne-Apelt.

Cells - 32 per c.mm. - practically all lympho-:cytes.

CONGENITAL HEART DISEASE

Cases - Fifteen.

History - All positive.

Clinical examination - All positive.

Lange reaction - Paretic curve in 2; luetic curve in 13.

Wassermann reaction - Positive with serum and cerebro-spinal fluid.

Globulin tests - All positive.

Foam test - All positive.

Cells - Varied between 19 and 100 per c.mm.

Small lymphocytes averaged	90	per	cent.
Large mononuclears	" 5·5	"	"
Plasma cells	" 1	"	"
Polymorphs	" 3	"	"
Eosinophils	" 0·5	"	"

CONGENITAL PYLORIC STENOSIS

Cases - Three; Positive 2; Negative 1.

History - Positive in 1 case.

Clinical examination - Positive in 1 case.

Lange reaction - Positive in 1 (luetic curve) and negative in 2 cases.

Wassermann reaction - Positive with serum and cerebro-spinal fluid in 2 cases and negative in 1.

Globulin/

Globulin tests - All positive in 1 case; all positive in another except Nonne-Apelt and Kaplan and all negative in the third.

Foam test - Positive in 2.

Cells - 0, 14 and 19 per c.mm. All small lympho-:cytes except 1 per cent. large mono-:nuclears.

ECZEMA ORIS

Cases - One.

History - Positive.

Clinical examination - Positive.

Lange reaction - Negative.

Wassermann reaction - Positive with serum and cerebro-spinal fluid.

Globulin tests - All positive.

Foam test - Positive.

Cells - 19 per c.mm. Practically all lymphocytes.

EPILEPSY

Cases - One.

History - Positive.

Clinical examination - Positive.

Lange reaction - Negative.

Wassermann reaction - Positive with serum and cerebro-spinal fluid.

Globulin tests - All positive except Ross-Jones.

Foam test - Positive.

Cells - 29 per c.mm. Practically all small lymphocytes.

GENERAL PARALYSIS

Cases/

Cases - Thirty-five, of which 10 were in the early stage and 2 were juvenile paralytics.

Lange reaction - Paretic type and positive in all except 2 of the early cases.

Wassermann reaction - Positive with cerebro-spinal fluid in all; negative in one serum.

Globulin tests - All positive.

Foam test - Positive in 31; not done in 4.

Cells - varied from 19 - 442 per c.mm.

Small lymphocytes ranged from	85	to	95	per	cent.
Large mononuclears	"	"	5	"	10	"	"
Plasma cells	"	"	1	"	3	"	"
Polymorphonuclears	"	"	0·5	"	1	"	"

HYDROCEPHALUS

Cases - Five.

History - Positive in 1 case.

Clinical examination - Positive in 2 cases.

Lange reaction - Very slight change in luetic zone in 4 instances, rising as far as 2 in the other case.

Wassermann reaction - Positive with serum and cerebro-spinal fluid in 3 and negative in 2 cases.

Globulin tests - Positive in 3 and negative in 2 cases.

Foam test - Positive in 3.

Cells - 0 and 1 per c.mm. in negative cases and 19, 30 and 68 in the positive. Cells mostly small lymphocytes with 1 to 5 per cent. plasma cells.

ICTERUS NEONATORUM

Cases/

Cases - One.

History - Positive.

Clinical examination - Positive.

Lange reaction - Luetic curve.

Wassermann reaction - Positive with serum and cerebro-spinal fluid.

Globulin tests - All positive.

Foam test - Positive.

Cells - 21 per c.mm. Practically all lymphocytes.

IDIOT WITH CONGENITAL SYPHILIS

Cases - One.

History - Positive.

Clinical examination - Positive.

Lange reaction - Paretic curve.

Wassermann reaction - Positive with serum and cerebro-spinal fluid.

Globulin tests - All positive.

Foam test - Positive.

Cells - 139 per c.mm.

Small lymphocytes	89·5	per cent.
Large mononuclears	7	" "
Plasma cells	2	" "
Polymorphonuclears	1·5	" "

IMBECILE WITH CONGENITAL SYPHILIS

Cases - Two.

History - Positive.

Clinical/

Clinical examination - Evidence of congenital syphilis.

Lange reaction - Luetic type.

Wassermann reaction - Positive with serum and cerebro-spinal fluid.

Globulin tests - Positive.

Foam test - Positive.

Cells - 19 and 33 per c.mm. Except one plasma cell, all are small lymphocytes.

INTERSTITIAL KERATITIS

Cases - Two.

History - Positive.

Clinical examination - Evidence of congenital syphilis.

Lange reaction - Luetic curve in one; Negative in other.

Wassermann reaction - Positive with serum and cerebro-spinal fluid.

Globulin tests - All positive, except Kaplan.

Foam test - Positive.

Cells - 15 and 67 per c.mm. Practically all lympho-:cytes.

MENINGITIS

Cases - Twenty-two.

Meningococcal - 9
Tubercular - 8
Syphilitic - 5

Meningococcal

Cases - Nine - Fluid slightly olive in 2; turbid in 7.

Lange/

Lange reaction - Negative in 9.

Wassermann reaction - Negative in 9.

Globulin tests - All positive with Pandy, Noguchi and sulphosalicylic mercuric-chloride. Other tests had each 5 positive results.

Foam test - All positive.

Cells - Only in 2 cases could cells be correctly enumerated owing to debris; in these cases cells were 1554 and 1320 per c.mm. Bulk of cells were polymorphonuclears with a few scattered mononuclears and eosinophils (0·5 per cent.).

It is interesting to observe that no fluid gave a meningitic curve nor any other type.

Tubercular meningitis

Cases - Eight. All were children under 4 years.

In one case fluid had a yellow tinge; in this case cells were :-

Polymorphonuclears	0·5 per cent.
Large mononuclears	0·5 " "
Plasma cells	0·5 " "
Lymphocytes	98·5 " "

In remainder of cases cells averaged :-

Small lymphocytes	50 per cent.
Large mononuclears	5 " "
Polymorphonuclears	45 " "

Lange reaction - Positive in 1; negative 7; in the positive the curve did not rise above 2 and is not a true meningitic curve.

Wassermann reaction - All negative.

Globulin tests - All positive in 2 and all negative in 3 cases. Partially positive with the remainder/

remainder.

Foam test - Positive in 7.

Syphilitic meningitis

Cases - Five. All cases had prolonged treatment, and were still under treatment at time of examination.

Lange reaction - Slight change in 2; negative in 3.

Wassermann reaction - Positive in 2; negative in 3.

Globulin tests - Positive in 3 and partially positive in 2.

Foam test - Positive in 5.

Cells - Varied from 10 to 18 per c.mm.

No polymorphonuclears nor large mononuclears seen: in 2 cases scanty plasma cells observed; remainder small lymphocytes.

Note: One month after all treatment had been suspended the three negative cases gave positive reactions.

SECONDARY ANAEMIA

Cases - Four.

History - Positive in 2; negative in 2.

Clinical examination - Doubtful in one case; not conclusive in other, but post-mortem examination leaves no room for doubt.

Lange reaction - Negative in 3; meningitic curve in other.

Wassermann reaction - Positive in 2 with serum and fluid.

Globulin tests - Slight increase in 1; not done in other positive case.

Foam/

Foam test - Positive in 1; not done in other.

Cells - 33 per c.mm. in one positive; chiefly lymphocytes; not done in other.

SYPHILIS

Cases - Thirty-nine.

Condylomata	- 2
Congenital	- 11
Latent	- 7
Neuro-	- 6
Primary	- 4
Secondary	- 9

Condylomata

Cases - Two.

History - Positive.

Clinical examination - Positive.

Lange reaction - Negative in both.

Wassermann reaction - Positive with serum and cerebro-spinal fluid in 2.

Globulin tests - Pandy and Sulphosalicylic-chloride - Positive in both; with Kaplan and Noguchi - Negative in one; positive in the other.

Foam test - Positive.

Cells - 9 and 14 per c.mm. All lymphocytes.

Congenital Syphilis

Cases - Eleven.

History - All positive.

Clinical examination - Positive in 2.

Lange/

Lange reaction	-	Luetic curve in all.
Wassermann reaction	-	Positive with serum and cerebro-spinal fluid in 11.
Globulin tests	-	Positive.
Foam test	-	Positive.

Cells - Varied from 12 - 39 ; chiefly lymphocytes.

Latent Syphilis

Cases	-	Seven.
History	-	Positive.
Clinical examination	-	Positive.
Lange reaction	-	Luetic curve in 2 but poor; Negative in 5.
Wassermann reaction	-	Positive with serum in 6 and with cerebro-spinal fluid in 5.
Globulin tests	-	Weakly positive in 6; not done in 1.
Foam test	-	Positive in 2; not done in 5.

Cells - Varied from 10 - 39. All small lymphocytes except an isolated plasma cell in a few instances.

Neuro-syphilis

Cases	-	Six.
History	-	Positive in 6.
Clinical examination	-	Negative in 2; Positive in 4.
Lange reaction	-	Paretic curve in 2; luetic curve in 1; Negative in 1; not done in 2.
Wassermann reaction	-	Positive with all cerebro-spinal fluids. Positive with 5 sera; doubtful with other.

Globulin/

Globulin tests - Positive in all.

Foam test - Positive in 4; not done in 2.

Cells - Varied between 13 and 100 per c.mm. Chiefly lymphocytes with 1 per cent. large mononuclears; no plasma cells.

Primary syphilis

Cases - Four.

History - Positive.

Clinical examination - Was positive but negative at time of tests.

Lange reaction - All negative.

Wassermann reaction - All negatives with serum and cerebro-spinal fluid.

Globulin tests - Not done.

Foam test - Not done.

Cells - Varied 0 - 1 per c.mm.

Secondary syphilis

Cases - Eleven.

History - Positive.

Clinical examination - Positive.

Lange reaction - Luetic curve in 4; negative in 7.

Wassermann reaction - Positive in 11 with serum and cerebro-spinal fluid.

Globulin tests - Partially positive.

Foam test - Positive in 8; not done in 3.

Cells - Varied from 9-64. Generally lymphocytes except in one case where polymorphonuclears were 15 per cent. and plasma cells 2 per.cent.

TABES/

TABES DORSALIS

Cases - Seven.

History - Positive.

Clinical examination - Positive.

Lange reaction - Luetic type. Positive in 6; Negative in 1.

Wassermann reaction - All positive.

Globulin tests - Noguchi and Pandy positive in 7.

Foam test - Positive in 7.

Cells - 12 - 22 per c.mm. Cells not greatly increased.

Small lymphocytes	average	93 per cent.
Large mononuclears	"	5 " "
Plasma cells	"	1·5 " "
Polymorphonuclears	"	0·5 " "

TRYPANOSOMIASIS

(This case is not included among those of syphilis)

Cases - One. Fluid supplied from London School of Tropical Medicine.

Patient became insane and died in Asylum.

Lange reaction - Rapid and complete flocculation in all tubes. A type which I have not seen described in the literature.

Wassermann reaction - Positive with serum and cerebro-spinal fluid.

Globulin tests - ALL strong.

Foam test - Positive.

Cells - 99 per c.mm.

Large mononuclears	-	0·5 per cent.
Plasma cells	-	0·5 " "

Remainder - Small lymphocytes except 2 isolated/

isolated non-nuclear mulberry-like cells.

NON-SYPHILITIC CASES

Two hundred and thirty cases are included in this section of the investigation. There was no history of syphilis, no evidence of it on clinical examination, and the Wassermann reaction with serum and cerebro-spinal fluid was negative in every in-:stance. The colloidal gold test, the globulin tests and the foam test gave positive results in a number of diseases which are included in the table below :-

TABLE

No.of Cases	Disease	Wasser-:mann Reaction		Lange	Noguchi	Ross-Jones.	Nonne-Apelt.	Pandy	Kaplan	Sulpho-salicylic acid	Mercuric Chloride	Foam
		Serum	Fluid									
4	Chronic Nephritis	–	–	2	–	–	–	–	–	–	–	–
4	Dental abscess	–	–	1	–	–	–	–	–	–	–	–
4	Diphtheria	–	–	1	–	–	–	–	–	–	–	–
18	Encephalitis Lethargica	–	–	16	13	12	8	17	9	17	17	17
2	Gonorrhœa	–	–	1	–	–	–	–	–	–	–	–
2	Infantile Paralysis	–	–	1	–	–	–	1	1	1	1	1
	INFLUENZA	–	–	2	–	–	–	–	–	–	–	–
21	Mild											
11	Late	–	–	11	11	6	6	11	6	11	11	11
4	Post			4	3	3	3	3	3	3	3	3
1	Severe	–	–	1	1	1	1	1	1	1	1	1
	MENINGITIS											
9	Acute	–	–	–	9	5	5	9	5	9	9	9
8	Tubercular	–	–	1	4	2	1	5	3	4	4	7
5	Mumps	–	–	1	–	–	–	–	–	–	–	–
10	Acute Rheuma-:tism	–	–	3	–	–	–	–	–	–	–	–
93				45	41	29	24	47	28	46	46	49

Thus/

Thus, with the exception of chronic nephritis, dental abscess, diphtheria, gonorrhœa, infantile para-:lysis, mild influenza, mumps and acute rheumatism which alone gave reactions with colloidal gold, there is a certain amount of agreement.

MUMPS

A search through the literature of this and other countries shows that pleocytosis may occur in the cerebro-spinal fluid in mumps. Lavergne (1917) reports the case of a child of 9½ years with tonsill-:itis and convulsions where polymorphs and lymphocytes were present in equal numbers. Feiling (1913) had a case with 2,500 cells per c.mm. with -

Lymphocytes	-	96 per cent.
Polymorphs	-	2 " "
Large hyaline	-	2 " "

Tasker-Howard (1919) had 3 cases with 2,500, 703 and 360 cells respectively of which 90 - 100 per cent. were mononuclears. Monod, so long ago as 1902, did systemic lumbar puncture in cases of mumps and found pleocytosis. He considered that a meningeal reaction was present in every case at some stage of the disease. De Massary, Tocmann and Luce (1917) examined the cerebro-spinal fluid of 56 soldiers suffering from mumps and found a pleocytosis in all but 7, and of these only 16 showed meningeal symptoms. Howard,/

Howard, Tessier and Essmein (1919) have observed a diplococcus in cerebro-spinal fluid in parotitis.

The number of cases examined by the writer was small.

Cases	-	Five.
Clinical examination	-	One in early stage and 4 convalescent.
Lange reaction	-	4 were negative and the one in the early stage gave a luetic curve.
Globulin tests	-	All negative.
Foam test	-	Negative in 5.

Cells - 2 cases had no cells; 2 had each 2, while 1 had 305 per c.mm.

INFANTILE PARALYSIS

Changes in the cerebro-spinal fluid vary with the stage of the disease. In the preparalytic stage, pleocytosis is a constant feature.

Cases	-	Two.
Clinical examination	-	Disease well established in both patients.
Lange reaction	-	1 negative; other showed a very slight mid zone change.
Globulin tests	-	1 negative - Kaplan, Pandy, Sulphosalicylic acid and Mercuric-chloride positive in other case.
Foam test	-	Positive in 1 and negative in the other.

Cells - /

Cells - 0 in one and 17 per c.mm. in other of which 98 per cent. was lymphocytes and 2 per cent. large hyaline.

CHRONIC NEPHRITIS

Cases - Four.

Clinical examination - Disease well established in 2 and advanced in 2.

Lange reaction - Negative in 2. In one there is a slight change in first 2 tubes and in the fourth an atypical change in tubes 2, 3, 4 and 5.

Globulin tests - All negative in 4.

Foam test - All negative.

Cells - 1, 2, 2 and 2 per c.mm.

DENTAL ABSCESS

Cases - Four.

Clinical examination - Slight swelling in 3; a large swelling in the fourth.

Lange reaction - Negative in 3; positive in 1, with a meningitic curve of moderate intensity.

Globulin tests - All negative in 4.

Foam test - All negative.

Cells - 1, 2, 3 and 6 per c.mm. The case which gave a colloidal gold reaction showed 1 cell per c.mm.

DIPHTHERIA

Cases - Four.

Clinical examination - All cases at the second day of treatment.

Lange/

Lange reaction - Negative in 3; positive with 1 which gave a meningitic curve.

Globulin tests - All negative in 4.

Foam test - All negative.

Cells - 0, 1, 2 and 3 per c.mm. The case which gave a Lange reaction had no cells per c.mm.

ACUTE RHEUMATISM

Cases - Ten.

Clinical examination - All cases showed some polysyno-:vitis.

Lange reaction - Positive in 3; negative in 7; in 2 there was a typical luetic curve while the third showed a slight change in the mid zone.

Globulin tests - All negative in 10.

Foam test - All negative

Cells - 0, 1 and 0 in the cases which gave a colloid-:al gold reaction.

ENCEPHALITIS LETHARGICA

Cases - Eighteen.

History - All positive.

Clinical examination - All patients had been serious-:ly ill for over 1 month but 1 case which gave negative results had been ill for over 3 months.

Lange reaction - Paretic curve in 15 and luetic in 1.

Globulin tests - Except in 1 negative case the tests were more or less positive.

Foam/

Foam test - Positive in 17 and negative in 1 where all other tests gave negative results and cells 4 per c.mm.

Cells - 4 and 23 in 2 negative cases; varied from 12 - 32 in 16 positive cases; in three instances only were cells over 20 per c.mm.

INFLUENZA

Cases - Thirty-seven.

Mild

Cases - Twenty-one .

Lange reaction - Atypical Lange curve in 2 showing changes in first four tubes in one instance and in first three in other. All other tests are negative but cells are 4 and 2 per c.mm. in these 2 cases.

Late and severe

Cases - Twelve.

Lange reaction - Positive in 5.

Globulin tests - Noguchi, Pandy, Sulphosalicylic acid and mercuric-chloride tests - posi-:tive with 12; Ross-Jones, Nonne-Apelt and Kaplan positive with 7.

Foam test - Positive with 12.

Cells - Varied from 12 - 90 per c.mm. Pleocytosis consisted of the mixed type, with an average of 75 per cent. polymorphs, and the remaind-:er small lymphocytes and large hyaline in almost equal proportions.

Post

Cases - Four.

Clinical/

Clinical examination - The patients presented no definite symptoms but remained more or less neurasthenic and lethargic.

Lange reaction - Paretic curve in 4.

Globulin tests - All positive in 3; all negative in 1.

Foam test - Positive in 3; negative in 1.

Cells - 5 per c.mm. in negative case and all polymorphs; 98,101 and 115 in three cases. Polymorphs were 82 per cent. with large hyaline 15 per cent., the remainder being lymphocytes, eosinophils, granular corpuscles and macrophages.

Of the two hundred and thirty non-syphilitic cases, the colloidal gold test showed some change in 45 cases, the sulphosalicylic acid and the mercuric-chloride in 46 each, the Pandy in 47 and the Foam test in 49. The Noguchi test was positive in 41, Ross - Jones in 29, Kaplan in 28 and Nonne-Apelt in 24. Thus the Pandy, sulphosalicylic mercuric-chloride tests are practically equal and form Class I. - Noguchi, with a lower percentage of positive results falls into Class II. but the Ross-Jones, Nonne-Apelt and Kaplan in Class III. (vide supra).

The following table gives a summary of the findings with the various tests :-

TABLE

	Positive Reactions	Negative Reactions	Not tested
Wassermann reaction of serum	0	230	0
" " of C.S.F.	0	230	0
Lange " " "	45	185	0
Noguchi test with "	41	189	2
Ross-Jones test " "	29	201	2
Nonne-Apelt " " "	24	206	2
Kaplan " " "	28	202	2
Pandy " " "	47	183	2
Sulphosalicylic-mercuric-chloride test "	46	184	2
Foam test with "	49	181	2

DISCUSSION/

D I S C U S S I O N.

The majority of serologists consider that in Wassermann technique unheated cerebro-spinal fluid intensifies the reaction, and some believe that if too great amount is used in the test anticomplementary effects are elicited; on the other hand, complement and haemolysin (to sheeps red blood cells) are never present in normal fluids but can be demonstrated in the majority of pathological fluids, especially those of syphilitic origin. The fluid in this investigat-:ion was heated so as to ensure uniformity when com-:pared with the inactivated serum of the same patient and the results suggest that heating the cerebro-spinal fluid to 55° C. does not interfere materially with the diagnostic results.

The results of this investigation in syphilit-:ic conditions are in accord with those of most other workers and show that, in early infections, the cerebro-spinal fluid, like other body fluids, contains the Wassermann reacting substance soon after the disease becomes generalised; and the presence of a positive reaction with the cerebro-spinal fluid is probably not indicative of disease of the central nervous system in every instance. Browning and Mackenzie (1923), however,/

however, say that a positive reaction of the spinal fluid test indicates syphilis of the nervous system.

Cases under treatment are known to react negatively - especially in the case of the serum - but in my series the fluid was negative also. When treatment was suspended for a month the reactions again became positive both with serum and cerebro-spinal fluid. This is a rather important consider-:ation for clinicians and may help to explain, in part at least, some of the reasons for divergent results when the patient's serum, or fluid, is tested at regu-:lar intervals and the treatment is intermittent.

With regard to protein increase, this in-:vestigation was chiefly confined to the globulins and to a comparison between the various recognised tests. There is much need for further investigation concern-:ing the types of protein that are increased, and more accurate chemical methods for estimating the exact quantity; while more information is required with regard to the role of euglobulin, pseudo-globulin, fibrinogen, hæmolysin, complement, and, perhaps, albumin which, in its proportion to globulin, sometimes, according to Levinson, reaches 12 - 1 (in acute mening-:eal conditions) and 7 - 3 in general paresis. Such comparisons would probably prove to be not only an interesting investigation but might throw fresh light on/

on diagnostic methods. In my series the globulin findings, though varying somewhat with the different tests, agree in general with the findings of most other workers.

Reference has already been made in this paper to allowing the precipitates in the sulphosalicylic acid and mercuric-chloride tests to stand for 24 hours in order to compare the amounts. It was found that alkaloid and metallic precipitants behaved differently with tuberculous and meningococcal conditions. In tuberculous meningitis the precipitate with mercuric-chloride was never less than twice as heavy as that with sulphosalicylic acid, while in meningococcal meningitis the heavier precipitate was with the alkal-:oid. These results were constant; their importance is obvious and they confirm the findings of Tashiro and Levinson (1917). Their observation to the effect that general paralysis sometimes shows a greater pre-:cipitate with mercuric-chloride was also confirmed but as the result was not found to be invariable its diagnostic value has less significance.

Increase of protein may occur alone or it may be associated with cell increase. Any form of mening-:eal inflammation may cause this. In syphilitic cond-:itions protein and cells frequently show some degree of relationship though the rule is not absolute, ex-:cept perhaps in well established untreated cases of general/

general paralysis. It cannot be too strongly emphas-:ized that if workers are to have uniform results some standard method for cell enumeration must be accepted. In the French method the fluid is centri-:fugalized and a smear is made on a slide: the other method in common use is that of the counting-cell. Several varieties of chamber are in use,

(1) the Thoma-Zeiss
(2) the Neubauer
(3) the Nageotte
(4) the Glaubermann
(5) the Fuchs-Rosenthal.

The French method is far from accurate because the size of drop may vary; it is not possible even under the most favourable circumstances to ensure uniform thickness in the smear and it should be re-:membered that the velocity of the centrifuge and the shape of the tube affect the nature of the deposit; altogether it is a somewhat "rough and ready" method without much to recommend it except, perhaps, that the type of cell can be studied on the same slide. The Glaubermann and Fuchs-Rosenthal chambers give more accurate results than the Thoma-Zeiss. Precise cytological methods cannot be over-rated because we know that modern methods of treatment and frequent lumbar puncture, have the effect of reducing the cells, but while this is so there is a type of syphilis which defies reduction of cells to the normal. My results

are comparable with those of workers who have used the same methods or, at least, have clearly indicated the method used.

The value of the Lange colloidal gold reaction is probably over-estimated. It is of less diagnostic value than the Wassermann reaction and its routine application seems hardly justified. It would be unwise to use it as a substitute because other workers have shown that paretic and luetic curves are not confined to syphilitic conditions but have been obtained with encephalitis lethargica and disseminated sclerosis, while in my series acute rheumatism, influenza and mumps have to be added to the list. The results of this investigation show that in certain other non-syphilitic conditions, e.g. infantile paralysis and nephritis, the luetic curve is less definite yet definite enough to suggest that future investigators may be able to demonstrate there are non-syphilitic conditions, other than those found by the writer, which will lead to confusion if too much reliance is placed on this protein test.

SUMMARY/

SUMMARY and CONCLUSIONS.

A careful evaluation has been made of certain tests applied to the cerebro-spinal fluid in the labor-:atory diagnosis of neuro-syphilis - the Wassermann reaction, the Lange colloidal gold test and various other protein tests, and cell enumeration.

The Wassermann and Lange reactions of the cerebro-spinal fluid were carried out in a series of 350 cases of which 119 were syphilitic, while various globulin tests and cell enumeration were made on 329.

The Wassermann reaction with the serum and cerebro-spinal fluid was negative in the 230 cases where syphilis could be excluded. In the clinically syphilitic cases the Wassermann reaction gave positive results with the cerebro-spinal fluid in 95·65 per cent.; and with the serum in 94·78 per cent., while positive results with the colloidal gold test were obtained in 85·84 per cent. As regards globulin tests the Pandy, mercuric-chloride and sulphosalicylic acid gave approximately 95 per cent. positive results, the Noguchi, 86 per cent, and the Ross-Jones, Nonne-Apelt and Kaplan, 71 per cent.

Pleocytosis has also been investigated and the results are detailed.

As a result of this investigation it is con-:cluded/

concluded that -

(1) Among these tests the Wassermann reaction gives the highest percentage of positive results in known syphilitic cases and is of most diagnostic significance.

(2) The Lange colloidal gold reaction is definitely of less diagnostic value than the Wassermann reaction giving a much lower percentage of positive reactions in known syphilitic cases.

(3) The "paretic" and "luetic" types of the Lange reaction are frequently noted in certain non-syphilitic conditions, for example, among those studied, encephalitis lethargica, disseminated sclerosis, acute rheumatism, mumps and late influenza.

(4) The Lange reaction is, therefore, quite unreliable in the diagnosis of neuro-syphilis.

(5) In syphilitic conditions, among the globulin tests (Noguchi, Ross-Jones, Nonne-Apelt, Kaplan, Sulphosalicylic-mercuric-chloride) the Pandy, Sulphosalicylic acid and Mercuric-chloride tests approximate to the Wassermann reaction in the percentage of positive results obtained.

(6) The Noguchi, Ross-Jones, Nonne-Apelt and Kaplan tests yield in syphilitic conditions a much lower percentage of positives than the Wassermann reaction.

(7) In certain non-syphilitic conditions studied, all the protein tests, like the Lange test, may yield positive results.

(8) In the syphilitic conditions of the central nervous system studied, the cell enumeration was never less than 10 per c.mm. It is suggested that even such a low count is significant of a pathological state.

A large proportion of these cases was seen in connection with my official work as Deputy Commissioner on Lunacy, General Board of Control for Scotland, so that/

that clinical examination, recording of history and the diagnosis were carried out by at least one other medical man; indeed, only cases were accepted for the investigation in which there was entire agree-:ment as regards diagnosis. Certain cases except where stated were provided by medical men in general practice.

TABLE I./

TABLE I.

Diseases examined.

Adenitis	7
Anaemia (secondary) ..	4
Anasarca	1
Banti's disease . ..	1
Blepharitis	1
Bronchitis	1
Broncho-pneumonia ..	1
Cancer of breast and rectum	2
Carditis, acute ..	13
" scarlatinal	1
Cerebral gumma ..	1
Chorea	3
Condylomata	2
Congenital heart disease	15
" pyloric stenosis	3
Coryza	2
Dental abscess	4
Diphtheria	4
Diplopia	1
Eczema	1
" Capitis	1
" Oris	1
Encephalitis lethargica	18
Endocarditis, Rheumatic	3
Enteric fever	5
Enteritis	1
Epilepsy	1
Gastritis	3
General paralysis, early	10
" " juvenile	2
" " late	23
Gonorrhœa	2

Hæmatemesis/

TABLE I. (contd.)

Haematemesis	1
Hay fever	1
Hiccough, severe	1
Hydrocephalus	5
Icterus, catarrhal	3
" neonatorum ..	1
Idiot	1
Imbecile	2
Infantile paralysis ..	2
Influenza	21
" late	11
" post	4
" severe	1
Interstitial keratitis ..	2
Malaria	1
Measles	9
Melæna	1
Meningitis	5
" acute	9
" tubercular ..	8
Mumps	5
Nephritis, acute	1
" chronic	4
Ozœna	1
Otitis media	4
Pericarditis	1
Periostitis	1
Pharyngitis	1
Phthisis	2
Pleurisy	1
Pneumonia	3
Psoriasis	1
Rat-bite fever	1
Rheumatism, acute	10
Rhinitis (Atrophic) ..	1

Sarcoma/

TABLE I. (contd.)

Sarcoma	3
Syphilis, congenital ..	11
" latent	7
" neuro	6
" primary ..	4
" secondary ..	9
Scarlet fever	16
Tabes dorsalis	7
" mesenterica ..	7
Tetanus	1
Tetany	10
Tonsillitis	1
Trypanosomiasis	1
Typhus fever	1
Whitlow	1
Whooping cough	1
Total ..	350

TABLE II.

SYPHILITIC CASES.

Atrophic Rhinitis		1
Banti's disease		1
Cerebral gumma		1
Congenital heart disease		15
Congenital pyloric stenosis		2
Eczema oris		1
Epilepsy		1
General paralysis	23	
" " early	10	
" " juvenile	2	35
Hydrocephalus		3
Icterus neonatorum		1
Idiot		1
Imbecile		2
Interstitial keratitis		2
Secondary Anæmia		2
Syphilitic meningitis		5
Syphilis		
Condylomata	2	
Congenital	11	
Latent	7	
Neuro-	6	
Primary	4	
Secondary	9	39
Tabes dorsalis		7
Total		119

Included in this table are 4 cases of primary syphilis which were negative to all tests applied in this investigation.

December 6.

COLLOIDAL GOLD I.

Patient	Disease	Lange Reaction 1	2	3	4	5	6	7	8	9	10	Control	Wassermann Reaction C.S.F.	Serum
A.K.	General Paralysis	4	4	4	4	4	4	1	0	0	0	–	+	+
M.C.	Tetany	Negative										–	–	–
A.B.	Pneumonia	Negative										–	–	–
J.M.	Congenital Syphilis	0	0	1	3	3	2	0	0	0	0	–	+	+
K.A.	do.	0	0	0	1	2	4	2	1	0	0	–	+	+
M.A.	do.	0	1	2	2	3	1	1	0	0	0	–	+	+

December 7.

M.C.	Tetany	Negative										–	–	–
J.C.	do.	Negative										–	–	–
K.J.	do.	Negative										–	–	–
F.J.	do.	Negative										–	–	–
K.P.	do.	Negative										–	–	–
H.M.	do.	0	0	0	0	1	1	0	0	0	0	–	–	–

December 6.

Globulin Tests.						cells.	Foam
Noguchi	Ross-Jones	Nonne-Apelt	Kaplan	Pandy	Sulpho-salicylic-mercuric-chloride	per C.MM.	Test
+	+	+	+	+	+	98	+
–	–	–	–	–	–	3	–
–	–	–	–	–	–	2	–
+	+	+	+	+	+	16	+
+	+	+	+	+	+	12	+
+	+	+	+	+	+	18	+

December 7.

–	–	–	–	–	–	0	–
–	–	–	–	–	–	1	–
–	–	–	–	–	–	0	–
–	–	–	–	–	–	0	–
–	–	–	–	–	–	1	–
–	–	–	–	–	–	0	–

December 8.

COLLOIDAL GOLD I.

Patient	Disease	Lange Reaction 1 2 3 4 5 6 7 8 9 10	Control	Wassermann Reaction C.S.F.	Serum
K.B.	Secondary Syphilis	Negative	–	+	+
J.B.	Congenital Syphilis	2 3 4 4 4 2 1 0 0 0	–	+	+
C.M.	Congenital Syphilis	0 0 1 2 3 4 3 2 1 0	–	+	+
E.M.	Secondary Syphilis	0 1 2 4 4 3 1 0 0 0	–	+	+
P.McG.	Secondary Syphilis	Negative	–	+	+
Isa C.	General Paralysis	5 5 5 4 4 1 0 0 0 0	–	+	+

December 9.

B.C.	General Paralysis	5 5 5 5 4 1 0 0 0 0	–	+	+
L.Q.	Acute Meningitis	Negative	–	–	–
P.C.	Encephalitis Lethargica	Negative	–	–	–
E.A.	Neuro-Syphilis.	Negative	–	+	?
D.A.	Neuro-Syphilis	0 0 0 2 3 4 1 0 0 0	–	+	+
J.McG.	Secondary Syphilis	Negative	–	+	+

December 8.

Globulin Tests.						Cells	Foam
Noguchi	Ross-Jones	Nonne-Apelt	Kaplan	Pandy	Sulpho-salicylic-mercuric-chloride.	per C.MM.	Test.
+	–	–	+	+	–	13	+
–	+	+	–	+	+	29	+
–	–	–	+	+	+	18	+
+	–	–	–	+	+	9	+
–	–	–	–	+	+	10	+
+	+	+	+	+	+	179	+

December 9.

+	+	+	+	+	+	239	+
+	+	+	+	+	+	1320	–
–	–	–	–	–	–	4	–
–	–	–	–	+	+	96	+
–	–	–	+	+	+	100	+
–	–	–		+	+	22	+

December 13.

COLLOIDAL GOLD II.

Patient	Disease	Lange Reaction 1 2 3 4 5 6 7 8 9 10	Control	Wassermann Reaction C.S.F.	Serum
Isabella C.	Encephalitis Lethargica	5 5 5 5 5 5 1 0 0 0	–	–	–
J.K.	Tubercular Meningitis	Negative	–	–	–
J.L.	do.	Negative	–	–	–
G.K.	Condylomata	Negative	–	+	+
G.N.	do.	Negative	–	+	+
I.D.S.	Interstitial Keratitis	0 0 1 3 3 3 2 0 0 0	–	+	+
O.R.	do	Negative	–	+	+

December 15.

John B.	General Paralysis	5 5 5 5 4 1 0 0 0 0	–	+	+
James G.	do.	5 5 5 4 3 0 0 0 0 0	–	+	+
Frank K.	do.	5 5 4 4 1 0 0 0 0 0	–	+	+
Joshua R.	do.	5 5 5 5 3 2 1 0 0 0	–	+	+
William C.	do.	5 5 5 5 4 3 0 0 0 0	–	+	+
Peter B.	do.	5 4 4 3 2 2 1 0 0 0	–	+	+
Walter A.	do.	5 5 5 4 2 1 0 0 0 0	–	+	+
James McK.	do.	5 5 4 4 4 2 1 0 0 0	–	+	+
Mary McK.	do.	5 5 5 5 5 3 1 0 0 0	–	+	+
Jane O'F.	do.	5 5 5 5 4 3 2 1 0 0	–	+	+
Kate O'R.	do.	5 5 5 5 4 3 3 2 1 0	–	+	+
Bridget H.	do.	5 5 5 4 4 3 2 1 1 0	–	+	+

December 13.

Globulin Tests						Cells per C.MM.	Foam Test.
Noguchi	Ross-Jones	Nonne-Apelt	Kaplan	Pandy	Sulpho-salicylic-mercuric-chloride.		
+	+	+	+	+	+	17	+
–	–	–	–	–	–	11	+
–	–	–	–	–	–	10	+
+	–	–	+	+	+	14	+
–	–	–	–	+	+	9	+
+	+	+	–	+	+	67	+
–	–	–	+	+	+	15	+

December 15.

+	+	+	+	+	+	59	+
+	+	+	+	+	+	120	+
+	+	+	+	+	+	45	+
+	+	+	+	+	+	104	+
+	+	+	+	+	+	238	+
+	+	+	+	+	+	74	+
+	+	+	+	+	+	52	+
+	+	+	+	+	+	82	+
+	+	+	+	+	+	261	+
+	+	+	+	+	+	190	+
+	+	+	+	+	+	220	+
+	+	+	+	+	+	114	+

December 15. (Continued)

COLLOIDAL GOLD II.

Patient	Disease	Lange Reaction 1 2 3 4 5 6 7 8 9 10	Control	Wassermann Reaction. C.S.F.	Serum
Jemima O'D.	General Paralysis	5 5 5 5 4 3 3 2 1 0	-	+	+
		December 16.			
Julia C.	Early General Paralysis	5 4 3 2 1 0 0 0 0 0	-	+	+
Edith McT.	do.	2 2 3 3 2 1 1 0 0 0	-	+	+
Hannah O'H.	do.	Negative	-	+	+
John McK.	do.	2 2 3 3 3 2 1 1 0 0	-	+	+
J.O.K.	do.	Negative	-	+	+
M.P.	do.	4 4 4 4 3 2 1 1 0 0	-	+	+
F.C.	do.	5 5 5 4 4 3 1 0 0 0	-	+	+
Jessie M.	do.	4 5 5 5 5 4 3 2 1 0	-	+	+
O.K.	do.	2 4 5 5 5 5 4 4 2 0	-	+	+
J.H.	do.	2 3 4 4 4 3 2 1 0 0	-	+	+
		December 17			
J.R.	Hydro-cephalus	0 0 1 1 0 0 0 0 0 0	-	-	-
T.P.	do.	0 0 1 2 1 1 1 0 0 0	-	-	-
Ann B.	Secondary Syphilis	0 0 4 4 3 3 2 1 1 0	-	+	+

December 15. (Continued)

Globulin Tests						cells.	Foam
Noguchi	Ross-Jones.	Nonne-Apelt.	Kaplan.	Pandy	Sulpho-salicylic-mercuric-chloride	per C.MM.	Test
+	+	+	+	+	+	105	+
			December 16.				
+	+	+	+	+	+	374	+
+	+	+	+	+	+	442	+
+	+	+	+	+	+	68	+
+	+	+	+	+	+	74	+
+	+	+	+	+	+	32	+
+	+	+	+	+	+	279	+
+	+	+	+	+	+	361	+
+	+	+	+	+	+	245	+
+	+	+	+	+	+	225	+
+	+	+	+	+	+	211	+
			December 17.				
-	-	-	-	-	-	1	-
-	-	-	-	-	-	0	-
-	-	-	-	-	+	18	+

December 17. (Continued)

COLLOIDAL GOLD II.

Patient.	Disease.	Lange Reaction. 1 2 3 4 5 6 7 8 9 10	Control	Wassermann Reaction C.S.F.	Serum
G.Q.	Secondary Syphilis	0 0 2 3 2 1 1 0 0 0	-	+	+
S.H.	do.	0 0 0 0 1 1 1 1 0 0	-	+	+
J.B.	Tertiary Syphilis	0 0 0 1 2 2 1 0 0 0	-	+	+

December 20.

Sarah B.	Acute Rheumatism	2 3 5 5 5 5 5 0 0 0	-	-	-
B.C.	do.	0 0 0 1 1 1 0 0 0 0	-	-	-
J.S.	do.	0 0 0 1 2 1 0 0 0 0	-	-	-
Kate McI.	do.	Negative	-	-	-
Abel C.	do.	Negative	-	-	-
Marion B.	do.	Negative	-	-	-
Clarinda S.	do.	Negative	-	-	-
Sybil W.	do.	Negative	-	-	-
W. W.	do.	Negative	-	-	-
J.K.	do.	Negative	-	-	-

COLLOIDAL GOLD III.

December 21.

J.B.	Encephalitis Lethargica.	5 5 5 5 5 4 3 2 1 0	-	-	-
I.C.	General Paralysis	5 5 5 5 4 3 2 1 0 0	-	+	+

December 17. (Continued)

Globulin Tests						Cells	Foam
Noguchi	Ross-Jones.	Nonne-Apelt.	Kaplan.	Pandy.	Sulpho-salicylic-mercuric-chloride.	Per C.MM.	Test.
-	-	-	-	+	+	22	+
+	-	-	-	+	+	12	+
+	+	+	-	+	+	16	+

December 20.

-	-	-	-	-	-	0	-
-	-	-	-	-	-	1	-
-	-	-	-	-	-	0	-
-	-	-	-	-	-	2	-
-	-	-	-	±	-	0	-
-	-	-	-	-	-	0	-
-	-	-	-	-	-	0	-
-	-	-	-	-	-	1	-
-	-	-	-	-	-	2	-
-	-	-	-	-	-	0	-

December 21.

+	-	-	-	+	+	18	+
+	+	+	+	+	+	270	+

December 21. (Continued)

COLLOIDAL GOLD III.

Patient.	Disease.	Lange Reaction 1 2 3 4 5 6 7 8 9 10	Control	Wassermann Reaction C.S.F.	Serum
R.L.	Hydrocephalus	0 1 1 0 0 0 0 0 0 0	-	+	+
P.R.	do.	0 1 1 1 0 0 0 0 0 0	-	+	+
H.L.	do.	0 0 1 1 1 1 0 0 0 0	-	+	+
H.D.	General Paralysis	5 4 4 4 4 4 4 1 0 0	-	+	+
J.H.	do.	5 5 5 5 5 5 4 3 2 0	-	+	+
Janet C.	Meningitis	0 0 0 0 0 0 0 1 0 0	-	+	+
Agnes K.	Meningitis	0 0 0 0 0 0 1 0 0 0	-	+	+
M.M.	Influenza	1 2 1 0 0 0 0 0 0 0	-	-	-
		December 22.			
Thomas W.	Tabes	0 2 2 1 0 0 0 0 0 0	-	+	+
J.C.D.	do.	0 0 3 2 1 0 0 0 0 0	-	+	+
James E.	do.	Negative	-	+	+
Patrick B.	do.	0 0 4 4 3 2 1 0 0 0	-	+	+
Alex. G.	do.	0 0 4 4 4 1 0 0 0 0	-	+	+
Peter G.	do.	0 0 3 4 3 2 1 0 0 0	-	+	+
E.H.G.	do.	0 0 4 5 5 1 0 0 0 0	-	+	+
		December 24:			
B.G.S.	Tetany	0 0 0 0 0 1 2 0 0 0	-	-	-
W.F.	Encephalitis Lethargica	5 5 5 5 5 4 2 0 0 0	-	-	-
C.J.W.	do.	5 5 5 5 4 3 2 1 0 0	-	-	-

December 21. (Continued)

Globulin Tests						Cells	Foam
Noguchi	Ross-Jones.	Nonne-Apelt.	Kaplan	Pandy	Sulpho-salicylic-mercuric-chloride.	per C.MM.	Test.
+	+	-	+	+	+	68	+
-	-	-	+	+	+	30	+
+	-	+	-	+	+	19	+
+	+	+	+	+	+	39	+
+	+	+	+	+	+	161	+
+	+	+	+	+	+	13	+
+	+	+	+	+	+	18	+
-	-	-	-	-	-	4	-
			December 22				
+	-	-	+	+	+	16	+
+	+	+	-	+	+	12	+
+	+	+	+	+	+	13	+
+	-	+	-	+	+	14	+
+	+	+	-	+	+	20	+
+	+	+	-	+	+	12	+
+	-	-	+	+	+	22	+
			December 24.				
-	-	-	-	-	-	0	-
-	+	-	-	+	+	16	+
+	-	-	-	+	+	17	+

December 24. (Continued)

COLLOIDAL GOLD III.

Patient.	Disease	Lange Reaction 1 2 3 4 5 6 7 8 9 10	Control	Wassermann Reaction C.S.F.	Serum
K.B.L.	Anaemia	0 0 0 0 0 2 3 2 1 1	-	+	+
Sarah N.	do.	Negative	-	-	-
Jessie B.	do.	Negative	-	-	-
Agnes W.	Acute Nephritis	Negative	-	-	-
Jeanie B.	Diphtheria	Negative	-	-	-
C.K.	Enteric Fever	Negative	-	-	-
L.C.K.	do.	Negative	-	-	-
K.C.H.	Scarlet Fever	Negative	-	-	-

December 25.

C.M.F.	Scarlet Fever	Negative	-	-	-
J.C.	do.	Negative	-	-	-
Peter C.	do.	Negative	-	-	-
James W.	Idiot	4 4 3 2 1 0 0 0 0 0	-	+	+
Kate B.	Imbecile	0 4 5 4 3 2 1 0 0 0	-	+	+
John C.K.	do.	0 0 4 4 3 2 1 0 0 0	-	+	+
A.C.H.	Juvenile General Paralysis	5 5 5 5 5 5 5 3 2 1	-	+	+
O.N.	do.	5 5 5 5 5 5 5 3 1 0 0	-	+	+

December 24. (Continued)

Globulin Tests.						Cells per C.MM.	Foam Test.
Noguchi	Ross-Jones.	Nonne-Apelt.	Kaplan.	Pandy.	Sulphosalicylic-mercuric-chloride.		
+	+	+	+	+	+	33	+
-	-	-	-	-	-	0	-
-	-	-	-	-	-	0	-
-	-	-	-	-	-	1	-
-	-	-	-	-	-	3	-
-	-	-	-	-	-	0	-
-	-	-	-	-	-	0	-
-	-	-	-	-	-	1	-

December 25.

-	-	-	-	-	-	1	-
-	-	-	-	-	-	0	-
-	-	-	-	-	-	0	-
+	+	+	+	+	+	139	+
+	+	+	+	+	+	33	+
+	-	-	+	+	+	19	+
+	+	+	+	+	+	42	+
+	+	+	+	+	+	19	+

December 26.

COLLOIDAL GOLD III.

Patient.	Disease	Lange Reaction 1 2 3 4 5 6 7 8 9 10	Control	Wassermann Reaction C.S.F.	Serum
C.F.	Measles	Negative	-	-	-
J.L.	do.	Negative	-	-	-
C.C.C.	do.	Negative	-	-	-
J.J.B.	do.	Negative	-	-	-
S.S.	do.	Negative	-	-	-
S.C.	do.	Negative	-	-	-
A.C.	do.	Negative	-	-	-
B.C.	do.	Negative	-	-	-
C.C.	do.	Negative	-	-	-

COLLOIDAL GOLD IV.

December 27.

O.D.C.	Congenital heart disease.	5 5 4 4 3 1 0 0 0 0	-	+	+
Agnes McW.	do.	0 0 5 5 2 1 0 0 0 0	-	+	+
Baby C.	do.	0 0 5 4 4 4 3 1 0 0	-	+	+
Baby L.M.	do.	0 0 0 3 3 3 1 1 0 0	-	+	+
Baby C.H.C.	do.	0 0 1 5 4 2 1 0 0 0	-	+	+
Marion G.	do.	0 0 3 5 5 1 0 0 0 0	-	+	+
Baby C. (Ayr)	do.	5 5 5 1 1 1 0 0 0 0	-	+	+
Baby Q. (Stirling)	do.	1 1 4 3 2 2 1 0 0 0	-	+	+
Baby P. (Dundee)	do.	2 2 3 3 1 1 1 0 0 0	-	+	+

December 26.

Globulin Tests.						Cells.	Foam
Noguchi	Ross-Jones.	Nonne-Apelt.	Kaplan	Pandy	Sulpho-salicylic-mercuric-chloride.	per C.MM.	Test.
-	-	-	-	-	-	0	-
-	-	-	-	-	-	1	-
-	-	-	-	-	-	0	-
-	-	-	-	-	-	3	-
-	-	-	-	-	-	2	-
-	-	-	-	-	-	1	-
-	-	-	-	-	-	2	-
-	-	-	-	-	-	1	-
-	-	-	-	-	-	2	-

December 27.

+	+	+	+	+	+	79	+
+	+	+	+	+	+	63	+
+	+	+	+	+	+	21	+
+	+	+	+	+	+	19	+
+	+	+	+	+	+	68	+
+	+	+	+	+	+	39	+
+	+	+	+	+	+	100	+
+	+	+	+	+	+	99	+
+	+	+	+	+	+	33	+

December 27. (Continued)

COLLOIDAL GOLD IV.

Patient.	Disease.	Lange Reaction 1 2 3 4 5 6 7 8 9 10	Control	Wassermann Reaction. C.S.F.	Serum
Baby McL. (Perth)	Congenital heart disease.	0 0 0 2 4 4 3 2 1 1	-	+	+
Baby O'F. (Perth)	do.	0 0 1 3 3 3 3 1 0 0	-	+	+
Mary C.S.	do.	0 0 2 4 3 3 2 2 1 0	-	+	+
J.J.	do.	0 3 4 5 5 4 3 1 1 0	-	+	+
W.W.W.W.	do.	0 0 1 3 4 5 2 1 0 0	-	+	+
A.L.K.	do.	0 1 3 5 4 3 2 1 0 0	-	+	+

December 28.

Agnes K.	Encephalitis Lethargica	5 5 5 5 5 5 4 3 1 0	-	-	-
J.J.C.	do.	5 5 4 4 3 2 1 1 0 0	-	-	-
S.J.	do.	5 4 4 4 3 2 1 1 0 0	-	-	-
W.F.	Congenital Pyloric Stenosis.	0 0 2 2 1 0 0 0 0 0	-	+	+
A.M.C.	do.	Negative	-	-	-
Jane N.	Broncho-pneumonia	Negative	-	-	-
Sarah M.	Pleurisy	Negative	-	-	-
J. McM.	Whooping Cough.	Negative	-	-	-
A.A.	Mumps	Negative	-	-	-
M.J.C.	Acute Carditis	Negative	-	-	-

December 27. (Continued)

Globulin Tests.						Cells.	Foam
Noguchi	Ross-Jones.	Nonne-Apelt	Kaplan	Pandy	Sulpho-salicylic-mercuric-chloride.	per C.MM.	Test.
+	+	+	+	+	+	32	+
+	+	+	+	+	+	36	+
+	+	+	+	+	+	28	+
+	-	+	+	+	+	19	+
+	-	-	+	+	+	37	+
+	+	+	+	+	+	19	+

December 28

+	-	-	+	+	+	18	+
+	-	+	-	+	+	17	+
+	+	+	+	+	+	22	+
+	+	+	+	+	+	14	+
-	-	-	-	-	-	0	-
-	-	-	-	-	-	1	-
-	-	-	-	-	-	0	-
-	-	-	-	-	-	0	-
-	-	-	-	-	-	0	-
-	-	-	-	-	-	1	-

December 28. (Continued)

COLLOIDAL GOLD IV.

Patient.	Disease	Lange Reaction 1 2 3 4 5 6 7 8 9 10	Control	Wassermann. Reaction C.S.F.	Serum
Annie M.P.	Scarlatinal Carditis	Negative	-	-	-
K.Y.	Rheumatic Endocarditis	Negative	-	-	-

December 29

Jane R.	Icterus Neonatorum	0 0 2 2 2 2 2 1 0 0	-	+	+
Annie F.Q.	Catarrhal Jaundice	Negative	-	-	-
J.O.	Enteritis	Negative	-	-	-
T.Y.	Banti's Disease	Negative	-	+	+
J.C.	Encephalitis Lethargica	5 5 5 5 4 4 3 1 0 0	-	-	-

COLLOIDAL GOLD V.

December 30.

L.L.	Gastritis	Negative	-	-	-
F.F.M.	do.	Negative	-	-	-
B.D.	do.	Negative	-	-	-
C.J.	Encephalitis Lethargica	5 5 5 5 4 3 2 2 1 0	-	-	-
E.F.	do.	4 3 3 2 2 1 1 1 0 0	-	-	-
G.R.	do.	3 3 3 2 2 1 1 0 0 0	-	-	-
S.F.	do.	3 3 3 3 2 1 0 0 0 0	-	-	-

December 28. (Continued)

Globulin Tests						Cells.	Foam
Noguchi	Ross-Jones	Nonne-Apelt	Kaplan	Pandy	Sulpho-salicylic-mercuric-chloride	Per C.MM.	Test.
-	-	-	-	-	-	0	-
-	-	-	-	-	-	0	-
			December 29.				
+	+	+	+	+	+	21	+
-	-	-	-	-	-	0	-
-	-	-	-	-	-	1	-
+	+	+	+	+	+	104	+
+	+	+	+	+	+	29	+
			December 30:				
-	-	-	-	-	-	0	-
-	-	-	-	-	-	3	-
-	-	-	-	-	-	0	-
+	-	-	-	+	+	32	+
-	+	+	-	+	+	12	+
-	+	-	-	+	+	12	+
+	-	+	+	+	+	10	+

December 30. (Continued)

COLLOIDAL GOLD V.

Patient.	Disease.	Lange Reaction 1 2 3 4 5 6 7 8 9 10	Control	Wassermann Reaction C.S.F.	Serum
P.R.	Encephalitis Lethargica	2 3 4 4 3 1 1 0 0 0	-	-	-
M.P.R.	Anasarca	Negative	-	-	-
Jane A.	Phthisis	Negative	-	-	-

December 31.

R.H.C.	Cancer	Negative	-	-	-
S.R.	do.	Negative	-	-	-
I.A.	Sarcoma	Negative	-	-	-
C.S.	Acute Meningitis	Negative	-	-	-
Flora W.	Diplopia	Negative	-	-	-
Cathie T.	Infantile Paralysis	Negative	-	-	-
M.N.	Jaundice	Negative	-	-	-
R.S.	Severe Hiccough	Negative	-	-	-
P.H.	Tetanus	Negative	-	-	-
D.P.	Endocarditis	Negative	-	-	-

January 2.

J.M.	Tabes Mesenterica	Negative	-	-	-
A.M.F.	do.	Negative	-	-	-

December 30. (Continued)

Globulin Tests						Cells.	Foam
Noguchi	Ross-Jones	Nonne-Apelt	Kaplan	Pandy	Sulpho-salicylic-mercuric-chloride	Per C.MM.	Test.
+	+	+	+	+	+	18	+
-	-	-	-	-	-	0	-
-	-	-	-	-	-	1	-
			December 31.				
-	-	-	-	-	-	0	-
-	-	-	-	-	-	0	-
-	-	-	-	-	-	1	-
+	+	+	+	+	+	numerous	+
-	-	-	-	-	-	0	-
-	-	-	-	-	-	2	-
-	-	-	-	-	-	0	-
-	-	-	-	-	-	3	-
-	-	-	-	-	-	2	-
-	-	-	-	-	-	0	-
			January 2.				
-	-	-	-	-	-	0	-
-	-	-	-	-	-	1	-

January 2. (Continued)

COLLOIDAL GOLD V.

Patient.	Disease	Lange Reaction 1 2 3 4 5 6 7 8 9 10	Wassermann Reaction Control	C.S.F.	Serum
A.R.	Tabes Mesenterica	Negative	-	-	-
Jane F.	do.	Negative	-	-	-
Maggie R.	do.	Negative	-	-	-
J.M.A.	do.	Negative	-	-	-
J.F.F.	do.	Negative	-	-	-
F.W.	Ozœna	Negative	-	-	-
Hannah H.	Meningitis	Negative	-	-	-
Mary C.B.	do.	Negative	-	-	-
Dorothy C.	do.	Negative	-	-	-

COLLOIDAL GOLD VI.

January 4.

Peter C.	Scarlet Fever	Negative	-	-	-
Claud P.	do.	Negative	-	-	-
Julia T.	do.	Negative	-	-	-
James S.	do.	Negative	-	-	-
John B.	do.	Negative	-	-	-
James M.	do.	Negative	-	-	-
Sam D.	do.	Negative	-	-	-
Leslie H.S.	do.	Negative	-	-	-
James S.	do.	Negative	-	-	-
Robert S.	do.	Negative	-	-	-
Ian S.	do.	Negative	-	-	-

January 2. (Continued)

Globulin Tests						Cells.	Foam
Noguchi	Ross-Jones	Nonne-Apelt	Kaplan	Pandy	Sulpho-salicylic-mercuric-chloride	Per C.MM.	Test.
-	-	-	-	-	-	2	-
-	-	-	-	-	-	0	-
-	-	-	-	-	-	0	-
-	-	-	-	-	-	3	-
-	-	-	-	-	-	2	-
-	-	-	-	-	-	0	-
+	+	+	+	+	+	18	+
+	-	-	-	+	+	12	+
+	+	-	-	+	+	10	+

January 4.

-	-	-	-	-	-	0	-
-	-	-	-	-	-	1	-
-	-	-	-	-	-	0	-
-	-	-	-	-	-	0	-
-	-	-	-	-	-	1	-
-	-	-	-	-	-	3	-
-	-	-	-	-	-	2	-
-	-	-	-	-	-	1	-
-	-	-	-	-	-	2	-
-	-	-	-	-	-	1	-
-	-	-	-	-	-	1	-

January 4. (Continued)

COLLOIDAL GOLD V.

Patient	Disease	Lange Reaction 1 2 3 4 5 6 7 8 9 10	Wassermann Reaction Control	C.S.F.	Serum
Mary S.	Scarlet Fever	Negative	-	-	-

January 5.

COLLOIDAL GOLD VI.

C.J.	Mumps	Negative	-	-	-
K.O.D.	do.	Negative	-	-	-
Jane I.	Encephalitis Lethargica	4 4 3 2 1 0 0 0 0 0	-	-	-
Mary S.	Influenza	1 2 1 1 0 0 0 0 0 0	-	-	-
J.M.C.	do.	Negative	-	-	-
A.L.McD.	do.	Negative	-	-	-
C.F.	do.	Negative	-	-	-
Mary Y.	Acute Endocarditis	Negative	-	-	-
Sam R.	Pericarditis	Negative	-	-	-

January 6.

B.C.	Influenza	Negative	-	-	-
Henry W.	Pyloric Stenosis	Negative	-	+	+
M.J.	Ichthyosis	Negative	-	+	+
C.M.J.	Eczema Capitis	Negative	-	-	-
Hugh K.	Eczema Oris	Negative	-	+	+
G.G.P.	Psoriasis	Negative	-	-	-
D.F.M.	Influenza	Negative	-	-	-
C.B.M.	Mumps	0 0 0 0 2 2 3 1 1 0 0	-	-	-

January 4. (Continued)

Globulin Tests						Cells.	Foam
Noguchi	Ross-Jones	Nonne-Apelt	Kaplan	Pandy	Sulpho-salicylic-mercuric-chloride	per C.MM.	Test.
-	-	-	-	-	-	3	-
			January 5.				
-	-	-	-	-	-	2	-
-	-	-	-	-	-	2	-
+	+	-	+	+	+	12	+
-	-	-	-	-	-	2	-
-	-	-	-	-	-	2	-
-	-	-	-	-	-	3	-
-	-	-	-	-	-	2	-
-	-	-	-	-	-	0	-
-	-	-	-	-	-	0	-
			January 6.				
-	-	-	-	-	-	3	-
+	+	-	-	+	+	29	+
+	-	+	-	+	+	16	+
-	-	-	-	-	-	2	-
+	+	+	+	+	+	19	+
-	-	-	-	-	-	0	-
-	-	-	-	-	-	2	-
-	-	-	-	-	-	305	-

January 6. (Continued)

COLLOIDAL GOLD VI.

Patient	Disease	Lange Reaction 1 2 3 4 5 6 7 8 9 10	Control	Wassermann Reaction C.S.F.	Serum
Fanny M	Infantile Paralysis	0 0 0 0 1 1 0 0 0 0	-	-	-
H. McQ.	Influenza	Negative	-	-	-
		January 7.			
S.V.	Influenza	Negative	-	-	-
T.T.	do.	Negative	-	-	-
C.V.	Epilepsy	Negative	-	+	+
D.P.	Acute Meningitis	Negative	-	-	-
F.Q.	Tubercular Meningitis	Negative	-	-	-
A.Y.	do.	Negative	-	-	-
		January 8.			
H.C.	Influenza	Negative	-	-	-
John D.	Carditis	Negative	-	-	-
E.E.	do.	Negative	-	-	-
Isa T.	Pneumonia	Negative	-	-	-
J.F.	do.	Negative	-	-	-
		January 10.			
M.C.	Coryza	Negative	-	-	-
Jean B.	Enteric Fever	Negative	-	-	-
Kate I.	do.	Negative	-	-	-

January 6. (Continued)

Globulin Tests						Cells.	Foam
Noguchi	Ross-Jones	Nonne-Apelt	Kaplan	Pandy	Sulpho-salicylic-mercuric chloride	per C.MM.	Test
-	-	-	+	+	+	17	+
-	-	-	-	-	-	2	-
			January 7.				
-	-	-	-	-	-	4	-
-	-	-	-	-	-	3	-
+	-	+	+	+	+	29	+
+	+	+	-	+	+	numerous	+
+	+	-	+	+	+	36	+
-	-	-	-	-	-	0	-
			January 8.				
-	-	-	-	-	-	2	-
-	-	-	-	-	-	0	-
-	-	-	-	-	-	3	-
-	-	-	-	-	-	2	-
-	-	-	-	-	-	5	-
			January 10.				
-	-	-	-	-	-	0	-
-	-	-	-	-	-	2	-
-	-	-	-	-	-	1	-

January 10. (Continued)

COLLOIDAL GOLD VI.

Patient	Disease	Lange Reaction 1 2 3 4 5 6 7 8 9 10	Control	Wassermann Reaction C.S.F.	Wassermann Reaction Serum
G.Y.	Enteric Fever	Negative	-	-	-
A.Q.	Congenital Syphilis	0 2 4 4 4 3 1 0 0 0	-	+	+
C.P.	do.	0 0 0 3 3 3 3 2 1 0	-	+	+

January 11.

A.A.	Typhus Fever	Negative	-	-	-
G.T.	Rat-bite Fever	Negative	-	-	-
Robina M.	Influenza	Negative	-	-	-

January 13.

K.N.	Influenza	Negative	-	-	-
B.B.A.	Congenital Syphilis	0 0 0 4 3 3 1 1 0 0	-	+	+
O.N.	do.	0 0 1 4 4 2 1 0 0 0	-	+	+
G.F.	do.	0 0 1 3 3 3 3 1 0 0	-	+	+

January 14.

C.C.	Coryza	Negative	-	-	-
D.A.	Severe Influenza	3 2 2 2 1 1 1 0 0 0	-	-	-
G.H.	Late Influenza	3 3 1 1 1 1 1 1 0 0	-	-	-

January 10. (Continued)

Globulin Tests						Cells.	Foam
Noguchi	Ross-Jones	Nonne-Apelt	Kaplan	Pandy	Sulpho-salicylic-mercuric-chloride	Per C.MM.	Test.
-	-	-	-	-	-	0	-
+	+	-	-	+	+	16	+
+	-	+	+	+	+	39	+
			January 11.				
-	-	-	-	-	-	0	-
-	-	-	-	-	-	3	-
-	-	-	-	-	-	6	-
			January 13				
-	-	-	-	-	-	6	-
+	+	-	-	+	+	39	+
+	-	+	+	+	+	20	+
+	-	+	+	+	+	23	+
			January 14.				
-	-	-	-	-	-	0	-
+	+	+	+	+	+	38	+
+	+	+	+	+	+	68	+

January 15.

COLLOIDAL GOLD VI.

Patient	Disease	Lange Reaction 1 2 3 4 5 6 7 8 9 10	Control	Wassermann Reaction C.S.F.	Serum
D.N.	Phthisis	Negative	-	-	-
R.R.	Dental Abscess	0 0 0 0 0 2 2 2 2 1	-	-	-
S.Y.	Cerebral Tumour	Negative	-	+	+
N.R.	Secondary Syphilis	Negative	-	+	+

January 16.

T.R.	Eczema	Negative	-	-	-
U.M.	Mumps	Negative	-	-	-
W.W.	Diphtheria	0 0 0 0 0 0 1 1 2 2	-	-	-
P.P.A.	do.	Negative	-	-	-
K.L.M.	do.	Negative	-	-	-

January 18.

N.C.	Influenza	Negative	-	-	-
W.K.	do.	Negative	-	-	-
Jane B.	do.	Negative	-	-	-
L.D.	do.	Negative	-	-	-
H.M.C.	Dental Abscess	Negative	-	-	-
Bella D.	do.	Negative	-	-	-
Flora A.	do.	Negative	-	-	-

January 15.

Globulin Tests						Cells.	Foam
Noguchi	Ross-Jones	Nonne-Apelt	Kaplan	Pandy	Sulpho-salicylic-mercuric-chloride	Per C.MM.	Test.
-	-	-	-	-	-	0	-
-	-	-	-	-	-	1	-
+	-	-	+	+	+	32	+
+	+	-	-	+	+	64	+

January 16.

-	-	-	-	-	-	1	-
-	-	-	-	-	-	0	-
-	-	-	-	-	-	0	-
-	-	-	-	-	-	1	-
-	-	-	-	-	-	2	-

January 18.

-	-	-	-	-	-	0	-
-	-	-	-	-	-	1	-
-	-	-	-	-	-	0	-
-	-	-	-	-	-	7	-
-	-	-	-	-	-	6	-
-	-	-	-	-	-	2	-
-	-	-	-	-	-	3	-

January 18. (Continued)

COLLOIDAL GOLD VI.

Patient	Disease	Lange Reaction 1 2 3 4 5 6 7 8 9 10	Wassermann Reaction Control	C.S.F.	Serum
N.R.	Acute Meningitis	Negative	-	-	-
R.R.A.	do.	Negative	-	-	-

February 21.

COLLOIDAL GOLD VII.

H.L.	Acute Meningitis	Negative	-	-	-
B.P.Q.	do.	Negative	-	-	-
O.McN.	do.	Negative	-	-	-
P. McA.	do.	Negative	-	-	-
G.J.	Tubercular Meningitis	Negative	-	-	-
H.C.	do.	0 0 0 0 0 1 2 2 1 0	-	-	-
George M.	do.	Negative	-	-	-
Ann O'R.	do.	Negative	-	-	-

February 22.

M.R.	Late Influenza	Negative	-	-	-
J.W.	do.	Negative	-	-	-
Clarus M.	do.	Negative	-	-	-

February 23.

C.F.	Late Influenza	2 2 2 2 2 1 1 0 0 0	-	-	-

January 18. (Continued)

Globulin Tests						Cells	Foam
Noguchi	Ross-Jones	Nonne-Apelt	Kaplan	Pandy	Sulpho-salicylic-mercuric-chloride	Per C.MM.	Test.
+	-	-	+	+	+	1554	+
+	+	+	+	+	+	numerous	+
				February 21.			
+	+	-	+	+	+	numerous	+
+	-	+	-	+	+	numerous	+
+	-	-	-	+	+	numerous	+
+	-	-	+	+	+	numerous	+
+	+	+	+	+	+	12	+
-	-	-	+	+	+	10	+
+	-	-	-	+	+	11	+
+	-	-	-	+	-	11	+
				February 22.			
+	+	+	+	+	+	35	+
+	+	+	+	+	+	61	+
+	+	-	-	+	+	53	+
				February 23.			
+	-	-	+	+	+	30	+

February 23. (Continued)

COLLOIDAL GOLD VII.

Patient	Disease	Lange Reaction 1 2 3 4 5 6 7 8 9 10	Control	Wassermann Reaction C.S.F.	Serum
Grace R.	Late Influenza	Negative	-	-	-
Bessie S.	do.	3 2 2 1 1 1 1 1 1 0	-	-	-
Bethia W.	do.	3 2 2 2 1 1 1 1 1 0	-	-	-
C.R.R.	do.	Negative	-	-	-
D.M.C.	Late Influenza	Negative	-	-	-
F.F.J.	do.	Negative	-	-	-
Annie K.	Post Influenza	3 3 3 2 2 1 0 0 0 0	-	-	-
Letitia B.	do.	3 3 3 3 2 2 2 0 0 0	-	-	-
Davidina Y.	do.	3 3 3 3 3 1 1 0 0 0	-	-	-
		February 25.			
I.J.	Chorea	Negative	-	-	-
K.K.Y.	do.	Negative	-	-	-
L.M.	do.	Negative	-	-	-
		February 26.			
P.C.	Influenza	Negative	-	-	-
		February 27.			
A.Q.	Influenza	Negative	-	-	-
C.L.C.	do.	Negative	-	-	-

February 23. (Continued)

Globulin Tests						Cells.	Foam
Noguchi	Ross-Jones	Nonne-Apelt	Kaplan	Pandy	Sulpjo-salicylic-mercuric-chloride	Per C.MM.	Test
+	-	-	-	+	-	12	+
+	+	+	+	+	+	14	+
+	-	+	-	+	+	90	+
+	+	+	-	+	-	64	+
+	-	-	-	+	+	15	+
+	-	-	+	+	+	39	+
+	+	+	+	+	+	101	+
+	+	+	+	+	+	98	+
+	+	+	+	+	+	115	+
			February 25.				
-	-	-	-	-	-	3	-
-	-	-	-	-	-	2	-
-	-	-	-	-	-	0	-
			February 26.				
-	-	-	-	-	-	3	-
			February 27.				
-	-	-	-	-	-	2	-
-	-	-	-	-	-	0	-

March 22.

COLLOIDAL GOLD VII.

Patient	Disease	Lange Reaction. 1 2 3 4 5 6 7 8 9 10	Control	Wassermann Reaction C.S.F.	Serum
P.S.	Adenitis	0 0 0 0 0 2 2 2 0 0	-	-	-
John R.	do.	Negative	-	-	-
Sam B.	do.	Negative	-	-	-

March 23.

B.Y.	Adenitis	Negative	-	-	-
R.J.	do.	Negative	-	-	-
P.R.	do.	Negative	-	-	-
R.S.	do.	Negative	-	-	-

March 24.

Flora H.	Tonsillitis	Negative	-	-	-
E.R.	Encephalitis Lethargica	Negative	-	-	-
G.G.	do.	1 2 3 4 1 1 1 0 0 0	-	-	-
A.L.	do.	2 2 2 2 1 0 0 0 0 0	-	-	-

COLLOIDAL GOLD VIII.

March 25.

S.J.	Trypanosomiasis	5 5 5 5 5 5 5 5 5 5	-	+	+
J.D.	Pharyngitis	Negative	-	-	-
G.B.	Rhinitis	Negative	-	+	+
A.A.	Blepharitis	Negative	-	-	-

March 22.

Globulin Tests						Cells.	Foam
Noguchi	Ross-Jones	Nonne-Apelt	Kaplan	Pandy	Sulpho-salicylic-mercuric-chloride	Per C.MM.	Test.
-	-	-	-	-	-	1	-
-	-	-	-	-	-	2	-
-	-	-	-	-	-	0	-
			March 23.				
-	-	-	-	-	-	0	-
-	-	-	-	-	-	0	-
-	-	-	-	-	-	1	-
-	-	-	-	-	-	0	-
			March 24.				
-	-	-	-	-	-	0	-
-	+	-	+	+	+	23	+
+	+	+	-	+	+	16	+
+	+	-	+	+	+	13	+
			March 25.				
+	+	+	+	+	+	99	+
-	-	-	-	-	-	0	-
+	-	-	+	+	+	20	+
-	-	-	-	-	-	2	-

March 25. (Continued)

COLLOIDAL GOLD VIII.

Patient	Disease	Lange Reaction 1 2 3 4 5 6 7 8 9 10	Control	Wassermann Reaction C.S.F.	Serum
L. McT.	Malaria	Negative	-	-	-
J. McG.	Influenza	Negative	-	-	-
		March 26.			
E.J.	Otitis Media	Negative	-	-	-
F.M.	do.	Negative	-	-	-
B.O.	do.	Negative	-	-	-
H.McI.	do.	Negative	-	-	-

COLLOIDAL GOLD IX.

		June 14.			
T.H.	Haematemesis	Negative	-	-	-
George F.	Melæna	Negative	-	-	-
Bessie C.	Post Influenza)	3 3 3 2 1 1 0 0 0 0	-	-	-
		June 21.			
D.R.	Bronchitis	Negative	-	-	-
L.L.Q.	Hay Fever	Negative	-	-	-
John L.	Carditis	Negative	-	-	-
James R.	do.	Negative	-	-	-

March 25. (Continued)

Globulin Tests						Cells.	Foam
Noguchi	Ross-Jones	Nonne-Apelt	Kaplan	Pandy	Sulpho-salicylic-mercuric-chloride	Per C.MM.	Test
-	-	-	-	-	-	6	-
-	-	-	-	-	-	6	-
			March 26.				
-	-	-	-	-	-	1	-
-	-	-	-	-	-	0	-
-	-	-	-	-	-	2	-
-	-	-	-	-	-	0	-
			June 14.				
-	-	-	-	-	-	2	-
-	-	-	-	-	-	3	-
-	-	-	-	-	-	5	-
			June 21.				
-	-	-	-	-	-	0	-
-	-	-	-	-	-	0	-
-	-	-	-	-	-	4	-
-	-	-	-	-	-	3	-

August 24.

COLLOIDAL GOLD X.

Patient	Disease	Lange Reaction 1 2 3 4 5 6 7 8 9 10	Wassermann Reaction Control	C.S.F.	Serum
T.S.H.	Carditis	Negative	-	-	-
Henry R.	do.	Negative	-	-	-
George B.	do.	Negative	-	-	-
		August 25.			
F.Q.	Periostitis	Negative	-	-	-
C. McG.	Carditis	Negative	-	-	-
L.B.	do.	Negative	-	-	-
		August 26.			
S.N.	Nephritis	1 1 0 0 0 0 0 0 0 0	-	-	-
Jane P.	do.	0 1 1 1 1 0 0 0 0 0	-	-	-
Jeanie McB.	do.	Negative	-	-	-
James L.	do.	Negative	-	-	-
		August 28.			
D.P.	Carditis	Negative	-	-	-
		August 29.			
L.J.	Carditis	Negative	-	-	-
Sarah W.	do.	Negative	-	-	-

August 24.

Globulin Tests						Cells.	Foam
Noguchi	Ross-Jones	Nonne-Apelt	Kaplan	Pandy	Sulpho-salicylic-mercuric-chloride	Per C.MM.	Test.
-	-	-	-	-	-	3	-
-	-	-	-	-	-	2	-
-	-	-	-	-	-	2	-

August 25

-	-	-	-	-	-	0	-
-	-	-	-	-	-	2	-
-	-	-	-	-	-	1	-

August 26.

-	-	-	-	-	-	2	-
-	-	-	-	-	-	1	-
-	-	-	-	-	-	2	-
-	-	-	-	-	-	2	-

August 28.

-	-	-	-	-	-	1	-

August 29.

-	-	-	-	-	-	3	-
-	-	-	-	-	-	2	-

August 30.

COLLOIDAL GOLD X.

Patient	Disease	Lange Reaction 1 2 3 4 5 6 7 8 9 10	Control	Wassermann Reaction C.S.F.	Serum
J.B.	Whitlow	Negative	-	-	-
H.H.M.	Tetany	Negative	-	-	-

August 31.

A.A.T.	Sarcoma	Negative	-	-	-
William T.	do.	Negative	-	-	-
C.H.H.	Congenital Syphilis	0 0 0 3 3 3 3 2 1 0	-	+	+
L.B.J.C.	Catarrhal Jaundice	Negative	-	-	-
S.H.	Tetany	Negative	-	-	-

August 30.

Globulin Tests.						Cells Per C.MM.	Foam Test.
Noguchi	Ross-Jones	Nonne-Apelt	Kaplan	Pandy	Sulphosalicylic-mercuric-chloride.		
-	-	-	-	-	-	3	-
-	-	-	-	-	-	2	-

August 31.

-	-	-	-	-	-	1	-
-	-	-	-	-	-	2	-
+	-	-	+	+	+	14	+
-	-	-	-	-	-	3	-
-	-	-	-	-	-	2	-

Incomplete tests were made on the following at various dates.

Patient	Disease	Lange Reaction 1	2	3	4	5	6	7	8	9	10	Control	Wassermann Reaction C.S.F.	Serum
Dr. Wilkie's case.	Neuro-Syphilis)	Not done											+	+
9401	General Paralysis)	4	5	5	5	5	4	2	1	0	0	-	+	+
Richmond	Gonorrhoea	Negative											-	-
7380	Primary Syphilis)	Negative											-	-
1430	General Paralysis)	5	5	5	4	4	2	1	0	0	0	-	+	-
Bruce	Gonorrhoea	0	1	3	4	4	4	3	1	0	0	-	-	-
A287	Latent Syphilis)	Negative											+	+
A1611	Primary Syphilis)	Negative											-	-
A1637	Primary Syphilis)	Negative											-	-
8720	Latent Syphilis)	Negative											-	-
Riddell	General Paralysis)	5	5	5	5	5	4	3	2	1	0	-	+	+
A1652	Latent Syphilis)	Negative											+	+
A1659	Syphilis of Central Nervous System)	5	5	5	5	5	5	4	2	0	0	-	+	+
A1182	do.	5	5	5	5	5	4	3	1	0	0	-	+	+
3864	Secondary Syphilis)	3	3	3	3	2	1	0	0	0	0	-	+	+

Globulin Tests

Noguchi	Ross-Jones	Cells.
+	+	Not done
+	+	23
Not done		Not done
Not done		Not done
+	+	34
Not done		Not done
+	+	13
Not done		Not done
Not done		Not done
+	+	10
+	+	58
Not done		Not done
+	+	13
+	+	22
+	+	14

Patient	Disease	Lange Reaction 1 2 3 4 5 6 7 8 9 10	Control	Wassermann Reaction C.S.F.	Wassermann Reaction Serum
9750	Latent Syphilis	Negative		–	+
5705	Primary Syphilis	Negative		–	–
A308	Latent Syphilis	0 0 0 ½ 1 1 1 1 0 0	–	+	+
A1450	General Paralysis	5 5 5 5 5 5 4 2 0 0	–	+	+
Miller	Secondary Anaemia	Negative		+	+
Howeson	Neuro-Syphilis	Not done		+	+

Globulin Tests.

Noguchi	Ross-Jones	Cells
+	+	16
–	–	1
+	+	39
+	+	34
Not done		Not done
+	+	Not done

SECTION F.

THE SYPHILITIC FACTOR in MENTAL DISEASE.

INTRODUCTION

SECTION F.

THE SYPHILITIC FACTOR IN MENTAL DISEASE

I N T R O D U C T I O N

The rôle of syphilis in the causation of mental disease is of such importance that any investigation of the subject likely to elicit further data is obviously of great practical value, and available statistics relating to syphilis as an aetiological factor in mental conditions show discrepancies which indicate the need for such investigation on the widest possible basis.

In recent years the Wassermann serum reaction and the examination of the cerebro-spinal fluid by chemical and cytological methods have contributed greatly, by supplementing clinical data and personal histories of cases, towards the accurate diagnosis of mental and neurological conditions of syphilitic origin and have proved a valuable asset in the study of the part played by syphilitic infection generally in the pathology of the nervous system.

It is now generally accepted that the recognition of syphilis by purely clinical methods, even in the active stages of the disease, may present considerable diagnostic difficulty and in its latent phase the actual existence of the infection can only be demonstrated by laboratory diagnostic methods. In addition, serum diagnosis has contributed greatly to establishing/

establishing the syphilitic aetiology of pathological conditions whose causation had long been regarded as doubtful.

The syphilitic factor in such conditions as gen-:eral paralysis and locomotor ataxia is, of course, now well recognised and the Wassermann reaction has undoubtedly established the syphilitic origin of these conditions. In fact, in no other phase of syphilis, with the exception of the secondary stage, is a posit-:ive Wassermann reaction more constant than in general paralysis.

The value, therefore, of the Wassermann reaction in any systematic study of the relationship of syphil-:is to mental disease generally is undoubted.

For a number of years the author has been engaged in a study of the syphilitic factor in various patho-:logical conditions and more recently further attent-:ion has been specially directed to this ætological agent in mental disease. With the aid of sero-diagnostic methods, it is reasonable to suppose that in asylum populations a larger proportion of latent cases may be detected than was at one time possible and the mode of investigation proposed has been to carry out systematic serum tests in all cases in part-:icular asylums and correlate the results with person-:al histories and clinical data with a view to obtain-:ing in this way the maximum of relevant information.

The object of this paper is to record the results obtained/

obtained from such an investigation carried out in (a) the Manx Mental Hospital where 290 cases were studied and (b) the Argyll and Bute Mental Hospital where 388 cases were studied. These may be regarded as collectively representative of the general types of mental disease met with in public mental hospitals in this country but it is intended that, if possible, this communication should be followed by subsequent reports dealing with similar investigations in other selected asylums.

ASYLUM STATISTICS RELATING TO MENTAL DISEASE

ENGLAND.— In the thirty-first report of the Board of Lunacy it is stated that in 1876 venereal disease was recorded as a cause of mental disease in 0·4 per cent. of the total admissions, being 0·6 for men and 0·2 for women. Among private male patients the per-:centage was 1·6 and for paupers it was only 0·3 per cent. In the forty-fourth report the yearly averages between 1879 and 1888 were for men, 0·8, and for women, 0·2 per cent.; among male private patients 1·8 and male paupers 0·6 per cent. From 1888 to 1896 quin-:quennial averages are recorded but after 1907 acquir-:ed and congenital syphilis are no longer quoted to-:gether.

TABLE I.

	Private		Rate-aided	
	Male	Female	Male	Female
1879 - 1888	1·8	0·2	0·6	0·2 per cent.
1889 - 1892	2·5	0·2	1·1	0·4 " "
1893 - 1897	4·2	0·3	1·8	0·5 " "
1898 - 1902	6·5	0·3	3·2	0·8 " "
1903 - 1906	8·3	0·5	4·8	1·0 " "
1907	12·0	0·5	7·3	1·2 " "
1908 - 1912	11·8	0·7	9·0	1·3 " "

It will be observed that among the men the proportion is higher in the case of private than in pauper patients while the converse obtains in women. With newer methods of investigation it was found that among 79,761 pauper patients 10·3 per cent. of the males showed evidence of acquired syphilis and 1·6 of the females. The incidence among males in some English mental hospitals is shown below.

TABLE II./

TABLE II.

(a) With high percentage:-

	Per cent.
Portsmouth Mental Hospital ..	28·3
Plymouth " " ..	22·5
Croyden " " ..	20·4
London County" " ..	20·0
Newcastle " " ..	19·1
Cardiff " " ..	13·2
Middlesex " " ..	12·0

(b) With low percentage:-

Salop Mental Hospital ..	1·1
Denbigh " " ..	1·3
Derby " " County ..	2·0
Westmorland and Cumberland " ..	2·2
Suffolk " " ..	3·1
Leicester and " " Rutland ..	3·2

SCOTLAND.- Mackenzie (1913) submitted the sera of 786 asylum cases to the Wassermann test. Two hundred and thirty four had been diagnosed as suffering from general paralysis and 94 per cent. gave a positive re-:sult. Of 126 cases tested before examination or clinical diagnosis 62 per cent. were positive. In 64 cases of imbecility 25 per cent. were found posit-:ive/

positive; while of the remaining 362 who showed no evidence of syphilis 8·8 were positive.

Gilmour (1913) at the Glasgow District Asylum, Gartloch examined 129 cases of admissions and found 21 per cent. positive among the women and 35·8 per cent. among the men. Of 176 female cases, which did not include paralytics, 14·7 per cent. were positive; and, again excluding paralytics, among the men 18 per cent. of his 173 cases were positive. Mackenzie further reports that of 258 admissions to Glasgow District Asylum 54 patients reacted positively in the Wasser-:mann test; while for the same period there was not admitted to the Mental Hospital for the Counties of Argyll and Bute a single case of general paralysis in which infection did not appear to have been contracted outside of these counties.

THE INCIDENCE OF SYPHILIS IN THE COMMUNITY GENERALLY.

In any consideration of the incidence of syphilis among patients suffering from mental disease, it is of course necessary to correlate the results obtained with the facts regarding the incidence of the infect-:ion in the general population.

Since the introduction of the test by Wassermann, Neisser and Bruck (1906), there are now abundant statistics available for comparing the findings of the different workers.

White/

White (1914) in his evidence before the Royal Commission on venereal diseases, basing his estimate upon a consideration of all available statistics, came to the conclusion that in the United Kingdom there were annually about 114,000 fresh cases of syphilis. In 1919, D. Watson estimated that in Scotland alone there were 27,000 new cases of syphilis annually. The figure is probably too high; on the other hand, those of the Registrar General (for Scotland)are probably too low because an examination of his figures for the decade 1912 - 1921 shows that 2,011 deaths occurred - that is just over 200 a year were due to syphilis - and we find that about 75 per cent. of the deaths were children under one year of age and only 47 adults died of syphilis. It seems fairly certain that certification was at fault when out of a populat-:ion of 4¾ millions only 47 adults died of syphilis, and yet almost three times as many children are certi-:fied as dying from that cause.

Perhaps a better estimate could be made from the death rate for general paralysis and locomotor ataxia because it is recognised that of those who contract syphilis 2·5 to 3 per cent. develop general paralysis or locomotor ataxia.

Mattauschek and Pilcz (1921) traced the cases of 4,134 officers of the Austrian Army infected between 1880 and 1900. By 1912 some 4·8 per cent. had devel-:oped general paralysis and 2·7 per cent. developed tabes./

tabes. One has, however, to bear in mind that these diseases are more prevalent among men than women. Vedder (1918) states that in Denmark about 2·5 per cent. of cases that contract syphilis eventually die of general paralysis. If then we take 3 per cent. as representing the figure for those who eventually die of general paralysis or tabes, we may conclude that in Scotland the number of cases of syphilis per annum is in the region of 9,000.

The Royal Commission on venereal diseases (1916) puts the percentage at not less than 10 of the whole population of large cities, there being little differ-:ence between Scotland and England. Ferguson Watson (1912) investigated 331 cases of children and found 10 per cent. were syphilitic as evidenced by the Wassermann test. Elliot (1914) examined 130 cases of children at the City of Glasgow Fever Hospital and obtained 10 per cent. positive reactions; among the series only 4 showed clinical evidence of syphilis. Greig (1923) examined about 100 girls at an Industrial School in Dundee and obtained positive results in 73 per cent. All improved under antisyphilitic treat-:ment. Laird (1923) examined 287 imbecile children in Baldovan Institution, Dundee, and excluding all those who had manifested signs of syphilis, found 139 with a positive Wassermann reaction. Chislett (1911) in his examination of mentally defective children ob-:tained positive results in 45 per cent. Ferguson Watson (1913) examined the sera of 105 defective child-:ren/

children (a) under school age, (b) unfit for school by reason of their mental defect, and (c) markedly defective children seen while assisting Dr. Oswald, Glasgow Royal Asylum, with his investigation for the Royal Commission on the feeble-minded in 1907. Of the 105 cases examined 51 gave a positive Wassermann reaction - 48·5 per cent. - and of that number 13·4 per cent. showed stigmata. Among the series were 14 Mongols, 3 of whom were positive. Stevens (1916) found 6 positive in his series of 18 Mongols. De Stefano (1920) found 34 positive out of 46 Mongols examined.

SEROLOGICAL METHODS USED IN THE PRESENT INVESTIGATION

The sera were examined by the Wassermann test and also by the more recently introduced Flocculation re-:action. The methods were those used in the Bacter-:iology Department, University of Edinburgh, and full details are given in Sections A. and B.

RESULTS

The number of cases examined was 678 and it was arranged that the laboratory tests should be completed before the history and clinical data were known to the author.

Of the 678 cases examined 290 were from the Manx Mental Hospital and 388 from Argyll and Bute Mental Hospital. The comparative results in the case of the/

the Manx Hospital are shown below :-

TABLE III.

Manx Hospital	Number of cases examined	Positive both reactions	Negative both reactions	*W.R. + *F.T. -	W.R. - F.T. +
Male	141	23	117	0	1
Female	149	23	124	0	2
Total	290	46	241	0	3

One male and one female case of feeble-mindedness and one female case of manic depressive insanity were positive in the flocculation test and negative with the Wassermann reaction. The mental conditions of the remainder are classified in the table.

TABLE IV./

* W.R. = Wassermann Reaction.
F.T. = Flocculation Test.

TABLE IV.

	Positive reaction			Negative reaction			Total cases tested
	Male	Female	Total	Male	Female	Total	
Acute Confusional Insanity	-	-	-	3	1	4	4
Dementia ...	4	3	7	15	14	29	36
Dementia praecox	4	6	10	29	26	55	65
Epileptic insanity	1	2	3	7	4	11	14
Feeble-minded	6	7	13	32	38	70	83
" " with epilepsy	-	-	-	-	2	2	2
General paralysis	1	-	1	-	-	-	1
Mania ...	-	1	1	4	-	4	5
Manic depressive	3	2	5	9	16	25	30
Melancholia	1	-	1	4	4	8	9
Paranoia ...	3	2	5	12	18	30	35
Miscellaneous	-	-	-	2	1	3	3
TOTALS	23	23	46	117	124	241	287

Of the 290 cases examined 141 were men and 149 were women. Twenty-three of either sex gave positive results in both tests or 16·31 per cent. for male patients and 15·43 for females - an average of 15·87 per cent. If one case of general paralysis is ex-:cluded the percentage falls to 15·60 or to an average of/

of 15·51 per cent. for both sexes. A history of syphilis or clinical evidence was present in 3·7 per cent.

The table shows the result of the examination of the 388 cases in the Argyll and Bute Asylum.

TABLE V.

Argyll & Bute Asylum	Number of cases examined	Positive both reactions	Negative both reactions	**W.R.+ **F.T.-	W.R.- F.T.+	W.R.- *F.T.
Male	185	39	139	0	0	7
Female	203	33	169	0	1	0
TOTAL	388	72	308	0	1	7

*In these 7 cases classified as "weakly positive" a trace only of flocculation was noted with the 1 : 2 and 1 : 4 dilution of serum. While known negative sera do not exhibit any change even in a 1 : 2 dilution, it is difficult to assess at present the diagnostic significance of the weak reactions observed in these 7 cases.

**W.R. = Wassermann reaction.
F.T. = Flocculation test.

One female case of melancholia gave a negative Wassermann reaction and a positive Flocculation test. Seven male cases which gave negative Wassermann results were weakly positive in the Flocculation test. Four of these were cases of dementia praecox, two were feeble-minded and one was a case of confusional insanity. Three of the cases had previously been resident in Institutions while two were ex-soldiers. The mental/

mental conditions of the remainder are shown in the following table.

TABLE VI.

	Positive reaction			Negative reaction			Total cases tested
	Male	Female	Total	Male	Female	Total	
Acute confusional insanity ..	-	2	2	-	1	1	3
Dementia ..	7	2	9	22	44	66	75
Dementia praecox	15	6	21	45	41	86	107
Epileptic insanity	-	1	1	1	2	3	4
Feeble-minded	5	2	7	26	14	40	47
" " with epilepsy	-	-	-	-	3	3	3
General paralysis	2	1	3	-	-	-	3
Mania	1	2	3	2	4	6	9
Manic depressive	2	2	4	14	11	25	29
Melancholia ..	1	5	6	9	19	28	34
Paranoia ..	6	9	15	19	27	46	61
Miscellaneous ..	-	1	1	1	3	4	5
TOTALS	39	33	72	139	169	308	380

One hundred and eighty five men and 203 women were examined and 39 of the former and 33 of the latter gave positive results in both tests, giving percent-:ages of 21·08 and 16·25 respectively. If the cases of general paralysis are deducted, then the men are 20·00 and the women 15·76 per cent., or 17·88 average for/

for both sexes (18·66 if paralytics are included). One male case of mania seems to be an incipient case of general paralysis. The percentage of cases which gave a history of syphilis or showed clinical evidence was 2·83.

D I S C U S S I O N

All the figures available are not strictly com-:parable with mine. Those for England are not of recent date while those for Scotland deal with admiss-:ions only, and, therefore, may include a higher pro-:portion of recent infections, while my investigations deal principally with latent cases.

This investigation shows that, taking both sexes together for each hospital, the Argyll and Bute Mental Hospital would come 6th. on the list when compared with those shown in Table II. and the Manx Hospital would also be placed 7th. on the same list. The former, however, with 18·66 per cent. is comparable to Newcastle with 19·1 and the latter with 15·87 stands relatively nearer Cardiff which has 13·2 per cent. Therefore, taking the English standard, both Hospitals would be returned as with a high percentage.

In his examination of 786 cases of admission, Mackenzie had 62 per cent. among 126 cases which were examined by laboratory methods before diagnosis and examination/

examination had been made, and 8·8 per cent. in his 362 cases which showed no clinical evidence of syphil-:is. In a further series of 258 cases 20·93 per cent. were positive, which is 2·3 per cent. higher than that found at Lochgilphead and 5·1 per cent. above the Douglas Hospital. Gilmour's figures were 28·40 per cent. for both sexes in his examination of 129 admiss-:ions. It is of special interest to note that in both Argyll and Bute and in the Manx Hospitals general paralysis was rarely seen in the past but at the pres-:ent time there are 2 men and 1 woman in one hospital and 1 man in the other, percentages in both instances which are very low indeed, and at present one might have such difficulty in finding a correct explanation that it is better not to seek one.

S U M M A R Y

Manx Mental Hospital.— The number of cases examined was 290, of which 46 gave positive results - 23 men and 23 women: 241 were negative - 117 men and 124 women while one man and 2 women were positive with the Flocculation test and negative with the Wassermann reaction. The male patients gave positive results in 16·31 per cent. and the female 15·43.

Argyll & Bute Mental Hospital.— The number of cases examined was 388 - 185 men and 203 women, and 39 of/

of the former and 33 of the latter gave positive results. One hundred and thirty-nine men and 169 women gave negative results while one woman was positive with the Flocculation test and negative with the Wassermann reaction. Seven men gave a doubtful reaction with the Flocculation test which were negative with the Wassermann reaction. The same result was obtained when these cases were tested one month later. They were tested with 6 different antigens. The male patients gave positive results in 21·08 per cent. and the women in 16·25 per cent.

CONCLUSIONS/

CONCLUSIONS

1. In this series of cases studied, the Flocculation test, if not superior to the Wassermann reaction, has not been proved to be in any way inferior, and its application is much simpler.

2. The investigation suggests that while syphilis in mental hospitals is more common among men than among women, there is not such a wide difference as was at one time supposed.

3. By means of laboratory methods the number of positive results can be increased. The percentage has been raised by 12·1 in the case of the Manx patients and by 15·0 for the Scottish Hospital.

4. Better conclusions can be arrived at when a greater number of mental hospitals are available for comparison, especially if the observations are made by the same worker.

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